QUANTITATIVE TRAIT LOCI AFFECTING THE AGRONOMIC PERFORMANCE OF A *Sorghum bicolor* (L.) Moench RECOMBINANT INBRED RESTORER LINE POPULATION

A Dissertation

by

JORGE LUIS MORAN MARADIAGA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2003

Major Subject: Plant Breeding

QUANTITATIVE TRAIT LOCI AFFECTING THE AGRONOMIC PERFORMANCE OF A *Sorghum bicolor* (L.) Moench RECOMBINANT INBRED RESTORER LINE POPULATION

A Dissertation

by

JORGE LUIS MORAN MARADIAGA

Submitted to the Texas A&M University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Approved as to style and content by:

William Rooney (Chair of Committee)	Javier Betran (Member)
Robert Klein (Member)	J. Creighton Miller, Jr. (Member)
	Mark A. Hussey (Head of Department)

August 2003

Major Subject: Plant Breeding

ABSTRACT

Quantitative Trait Loci Affecting the Agronomic Performance of a *Sorghum bicolor* (L.) Moench Recombinant Inbred Restorer Line Population. (August 2003)

Jorge Luis Morán Maradiaga, B.S., Escuela Agricola Panamericana, Honduras;

M.S., Plant Breeding, Texas A&M University

Chair of Advisory Committee: Dr. William Rooney

Lately the rate of genetic gain in most agronomic crop species has been reduced due to several factors that limit breeding efficiency and genetic gain. New genetic tools and more powerful statistical analyses provide an alternative approach to enhance genetic improvements through the identification of molecular markers linked to genomic regions or QTLs controlling quantitative traits. The main objective of this research was to identify genomic regions associated with enhanced agronomic performance in lines per se and hybrid combination in *Sorghum bicolor* (L.) Moench. A population composed of 187 $F_{5:6}$ recombinant inbred lines (RIL) was derived from the cross of restorer lines RTx430 and RTx7000. Also, a testcross hybrid population (TCH) was developed by using each RIL as a pollinator onto ATx2752. A linkage map was constructed using 174 marker loci generated from AFLP and SSR primer combinations. These markers were assigned to 12 different linkage groups. The linkage map covers 1573 cM with marker loci spaced at an averaged 9.04 cM. In this study, 89 QTL that control variation in seven different morphological traits were identified in the recombinant inbred line population, while in the testcross hybrid population, 79 QTL were identified. These traits included

grain yield, plant height, days to mid-anthesis, panicle number, panicle length, panicle exsertion and panicle weight. These putative QTL explained from 4 to 42% of the phenotypic variation observed for each trait. Many of the QTL were not consistent across populations and across environments. Nevertheless, a few key QTL were identified and the source of the positive additive genetics isolated. RTx7000 was consistently associated with better agronomic performance in RIL, while in testcrosses, RTx430 was. Some genomic regions from RTx7000 may be utilized to improve RTx430 as a line per se. However, it is very unlikely that such regions will have a positive effect on the combining ability of RTx430 since testcross results did not reveal any transgressive segregants from the RIL population.

DEDICATION

To the one that departed my life early and to those who are coming to fill the void.

ACKNOWLEDGMENTS

I would like to thank Dr. Bill Rooney for allowing me to obtain my Ph.D. under his advisement. I will always be grateful to Dr. Javier Betran for taking time from his busy schedule to visit with me and help me with all my statistical analyses. My eternal gratitude to Dr. Monica Menz for guiding me through the completion of my research.

I am grateful to Dr. Patricia Klein for providing the funding to complete the genotyping of the population. Special thanks to Dr. Robert Klein and Dr. Creighton Miller for serving on my committee and for their invaluable input.

To Karen Prihoda and Cindy King for their friendship and constant support throughout these years. To Delroy Collins and the student workers for your invaluable help. To Rafael, Cleve, Gabriel, and Les for making my life at the Sorghum Breeding Program more enjoyable.

Finally, I would like to express my most sincere gratitude to Pioneer Hi-Bred International and its International Scholarship Committee for providing the funding that allowed me to complete my graduate studies at Texas A&M University.

TABLE OF CONTENTS

Page

ABSTRACT iii

DEDICATION v

ACKNOWLEDGMENTS vi

TABLE OF CONTENTS vii

LIST OF FIGURES x

LIST OF TABLES xi

CHAPTER

I INTRODUCTION 1

II REVIEW OF LITERATURE 4

Origin and History of Sorghum 4
Sorghum Genetics and Morphology 5
Sorghum Production 7
Sorghum Improvement 8
Molecular Breeding 10
Quantitative Trait Loci Mapping 11
Sorghum Linkage Maps 15
Quantitative Trait Loci in Sorghum 17
Quantitative Trait Loci Stability 21
Heterosis and QTL Mapping 24

III HERITABILITY ESTIMATES FOR GRAIN YIELD AND YIELD RELATED TRAITS IN A *Sorghum bicolor* (L.) Moench RECOMBINANT INBRED RESTORER LINE POPULATION 26

Introduction 26
Materials and Methods 28
Plant Materials 28
Experimental Design 30
Phenotypic Evaluation 31

CHAPTER Page

Statistical Analyses 33
Broad Sense Heritability Estimation 38
Phenotypic and Genotypic Correlation 40
Results and Discussion 41
GLM versus MIXED 41
Efficiency Analyses 41
Variances 42
Heritability Estimates 44
Adjusted Means 45
Trait Analyses 47
Grain Yield 47
Plant Height 51
Days to Mid-anthesis 54
Tillering Ability 59
Panicle Length and Exsertion 61
Panicle Weight 67
Trait Correlations 70
Conclusions 73

IV GENETIC MAPPING AND ANALYSIS OF QUANTITATIVE TRAIT LOCI IN SORGHUM FOR GRAIN YIELD AND SIX YIELD RELATED TRAITS 75

Introduction 75
Materials and Methods 77
Mapping Population 77
Genotypic Data 77
Anchor Molecular Markers 79
Amplified Fragment Length Polymorphism (AFLP) Marker Amplification 79
AFLP Marker Pre-Amplification and Adapter Ligation 79
Selective AFLP Amplification 80
Simple Sequence Repeat (SSR) Marker Amplification 82
Amplification Product Analysis 83
Linkage Map Construction 83
QTL Analyses 85
Linear Additive Model Selection 86
Results and Discussion 88
Linkage Map 88
QTL Analyses 91
Grain Yield 91

CHAPTER Page

Plant Height 99
Days to Mid-anthesis 107
Tillering Ability 113
Panicle Length 119
Panicle Exsertion 124
Panicle Weight 129
Conclusions 134

V SUMMARY 136

REFERENCES 139

APPENDIX 152

VITA 185

LIST OF FIGURES

FIGURE		Page
1	Sorghum yields in 7-year increment, 1950-1998	9
2	Putative grain yield QTL at individual and across environments	93
3	Putative plant height QTL at individual and across environments	100
4	Putative days to mid-anthesis QTL at individual and across environments	109
5	Putative tillering QTL at individual and across environments	114
6	Putative panicle length QTL at individual and across environments	120
7	Putative panicle exsertion QTL at individual and across environments	125
8	Putative panicle weight QTL at individual and across environments	130

LIST OF TABLES

TABLE		Page
1	Expected mean squares for the individual environment analysis of variance	35
2	Expected mean squares for the combined analysis of variance across environments	37
3	Efficiency of MIXED compared to GLM for seven traits evaluated in the RIL and TCH populations at different environments	42
4	Comparisons of the σ^2_G, σ^2_{GE} and σ^2_ε from the combined analyses of the RIL and TCH using the GLM and MIXED statistical procedures	43
5	Comparison of the broad sense heritability estimates (*H*) from the combined analyses of the RIL and TCH populations using the GLM and MIXED statistical procedures	45
6	Analysis of variance for grain yield (GYL) at individual and combined environments for RIL and TCH	48
7	Grain yield (GYL) and plant height (PHE) estimated parameters for the RIL and TCH populations by individual and combined environments	49
8	Analysis of variance for plant height (PHE) at individual and combined environments for RIL and TCH	53
9	Analysis of variance for DMA at individual and combined environments for RIL and TCH	55
10	Days to mid-anthesis (DMA) and tillering ability (PAN) estimated parameters for the RIL and TCH populations by individual and combined environments	58
11	Analysis of variance for PAN at individual and combined environments for RIL and TCH	60

TABLE Page

12 Analysis of variance for PLE at individual and combined environments for RIL and TCH 62

13 Analysis of variance component coefficients for PEX at individual and combined environments for RIL and TCH 63

14 PEX and PLE estimated parameters for the RIL and TCH populations by individual and combined environments 65

15 Analysis of variance for PWE at individual and combined environments for RIL and TCH 68

16 PWE estimated parameters for the RIL and TCH populations by individual and combined environments 69

17 RIL combined genotypic (r_G) and phenotypic (r_P) correlation coefficients among seven traits and their standard errors across three environments 71

18 TCH combined genotypic (r_G) and phenotypic (r_P) correlation coefficients among seven traits and their standard errors across three environments 72

19 *Eco*RI and *Mse*I primer combinations utilized to generate AFLP polymorphic markers..... 81

20 Primer sequence of SSR utilized as anchor markers in the RIL linkage map. 82

21 Linkage group designation (LG), recombinant length, and average cM distance between markers of the RIL linkage map 90

22 Peaks, LOD, R^2, and additive genetic effect of grain yield QTL in the RIL and TCH populations 92

23 RIL and TCH digenic epistatic interactions for grain yield across environments 96

24 Peaks, LOD, R^2, and additive genetic effect of plant height QTL in the RIL and TCH populations 102

TABLE Page

25 RIL and TCH digenic epistatic interactions for plant height across environments 105

26 Peaks, LOD, R^2, and additive genetic effect of days to mid-anthesis QTL in the RIL and TCH populations 108

27 RIL and TCH digenic epistatic interactions for days to mid-anthesis across environments 112

28 Peaks, LOD, R^2, and additive genetic effect of tillering QTL in the RIL and TCH populations 117

29 RIL and TCH digenic epistatic interactions for tillering across environments 118

30 Peaks, LOD, R^2, and additive genetic effect of panicle length QTL in the RIL and TCH populations 122

31 RIL and TCH digenic epistatic interactions for panicle length across environments 124

32 Peaks, LOD, R^2, and additive genetic effect of panicle exsertion QTL in the RIL and TCH populations 127

33 Peaks, LOD, R^2, and additive genetic effect of panicle weight QTL in the RIL and TCH populations 132

34 RIL and TCH digenic epistatic interactions for panicle weight across environments 133

CHAPTER I

INTRODUCTION

The success of a crop breeding program is based on the availability of genetic variability from which to select and the efficient utilization of such variability. Depending upon the scope of the program, the breeder might utilize exotic germplasm and/or utilize existing adapted germplasm as a source of different genes or genomic blocks that protect or improve potential yield, or in the best scenario, both.

Crop improvement programs have been very successful in improving the genetic yield potential of our major agronomic crop species (Troyer, 1999). While this improvement continues, the rate of gain in most species has been reduced, since genetic improvement is threatened by several factors that limit breeding efficiency and genetic gain (Maunder, 1999). Compared to a few years ago, germplasm selection is now being conducted in harsher environments; lower moisture availability, increased disease and insect pressure, and higher temperatures are among the environmental factors that have a direct impact on the agronomic performance of genetic material. Under such conditions and for quantitatively inherited traits such as grain yield, genetic variance declines, thus

---

This dissertation follows the style of Crop Science.

reducing the breeder's ability to differentiate superior from average performing germplasm.

Finding different genes in wild relatives to increase genetic gains has been explored in different crops (Zhuang et al., 1997; Wooten, 2001). Even though introgression of qualitative traits into elite germplasm has been successful, the same cannot be said about quantitative traits. Lack of fertile progeny, disruption of favorable linkage blocks and gene combinations, linkage drag, and time frame, are among the factors that have precluded the selection of superior genotypes for breeding purposes (Brondani et al., 2002).

An alternative approach to sustaining genetic variation in crop improvement programs is to utilize existing adapted germplasm and rely on the presence of *de novo* generation of genetic variation (Rasmusson and Phillips, 1997). In addition, this germplasm may contain valuable alleles that were not included in advanced germplasm during the breeding process. This germplasm has the added benefit of being adapted to the current production systems. This study represents an attempt to estimate the amount of genetic variability that may be exploited from the utilization of old elite lines to create new breeding populations, and to determine if any elite genes were left behind in these old genetic pools. It is of crucial importance to establish if such "old" genes can be useful in current attempts to increase genetic gains, and if so, determine if these genes will still perform well in hybrid combination.

New genetic tools, such as molecular markers, provide new alternative selection approaches to enhance genetic improvements in yield. Identification of molecular

markers linked to genomic regions or QTLs controlling grain yield as well as traits highly correlated to it, and their proper utilization, might enable breeders to establish marker assisted selection programs to increase the speed of backcross conversions, define a starting block of QTLs that should be present in any elite germplasm, and identify "small" effect QTLs that are not easily detected through conventional means.

The objectives of this research were (1) to estimate genetic parameters on seven different traits in a recombinant inbred restorer line population, (2) to identify genomic regions associated with grain yield in the recombinant population, (3) to determine which of the putative QTLs are associated with enhanced agronomic performance in lines per se and in hybrid combination, and (4) to determine which QTLs that are present in old elite germplasm might be introgressed into current elite germplasm for agronomic improvement.

CHAPTER II

REVIEW OF LITERATURE

Origin and History of Sorghum

Within *Sorghum* sp., is an incredible array of genetic diversity with which the cultivated sorghum, *Sorghum bicolor* L. Moench has the potential to exchange alleles for the improvement of the crop. The genus is classed into several wild and domesticated races. The cultivated races of *S. bicolor* are bicolor, guinea, kafir, caudatum and durra. The origin of these domesticated races has been associated with human migrations 3000 B.C. in Africa (Kimber, 2000), from where they migrated to Asia. The earliest sorghum in India dates back to the end of the third millennium (Meadow, 1996). While Qiao and Zhenshan (1970) believe sorghum was introduced to Southern China as a domesticated crop before 1045 B.C., some authors suggest that it was domesticated in northern China as early as 8500 B.C. (Kimber, 2000).

Early introductions of this crop to the United States occurred in 1853, when a sweet Chinese Amber sorghum was introduced from France (Martin, 1936). White and brown grain sorghums were later brought from Egypt to California in 1874 (Maunder, 1999). Several key introductions followed, including kafir in 1876, milo in 1879, feterita and hegari in 1906 and 1908, respectively. Early selections for improved varieties were

merely based on height and maturity, since early and dwarf milos were easier to harvest and appeared to have better drought tolerance compared to the tall types.

In the 1920s, with the application of the newly discovered principles of genetics, new improved dwarf cultivars with higher yields and better agronomic characteristics were developed through hybridization of kafir and milo germplasm (Swanson and Laude, 1951). Sorghum cultivars were developed that were harvestable using a wheat reaper and thresher. These genotypes were quickly adopted and predominated sorghum production throughout the Central Great Plains.

Based on the success of hybrid corn, early sorghum breeders knew the potential of hybrids, but had no means by which to economically produce seed (Conner and Karper, 1927). However, Stephens and Holland (1954) identified a cytoplasmic male-sterility system that would allow the cost-effective production of F_1 sorghum hybrids in the U.S. and the rest of the world (Maunder, 1999). Once hybrid sorghum seed was produced, it was rapidly accepted by sorghum producers and replaced sorghum cultivars in a period of less than ten years.

Sorghum Genetics and Morphology

Sorghum bicolor ($2n = 2x = 20$) is an annual C4 monocot. Sorghum is predominantly a self-pollinated species although outcrossing does occur at rates between 3 to 15%, depending on genotype and environment. Genetically, *S. bicolor* is a functional diploid crop although there is evidence of a tetraploid origin (Dogget, 1998).

There is some loci duplication (8 – 11%) and double probe hybridization (23%) in sorghum; however, these figures are not as high as in other crops of tetraploid origin such as maize (Dufuor, 1996). Using fluorescent *in situ* hybridization (FISH), Gomez et al., (1997) identified genomic probes that hybridized strongly and consistently to five of the ten chromosomes in *S. bicolor*, indicating that these five pairs of chromosomes may have a common evolutionary history unique from the other five pairs. Nevertheless, Peng et al., (1999) concluded that sorghum must remain a diploid from the perspective of genome organization and functionality, since there is not enough evidence to suggest otherwise. *Sorghum bicolor* has a nuclear DNA content of 1.55 to 10.6 pg per 2C or 748 to 772 million base pairs (Mbp) per 1C, which is three times smaller than the maize genome (2500 MB per 1C), 20 times smaller than the wheat genome (15966 Mbp per 1C), and double the nuclear content of the rice genome (450 Mbp per 1C) (Subudhi and Nguyen, 2000).

Genetic variation within the *Sorghum* subspecies is impressive. Plant heights vary from less than a meter to five meters; inflorescence types vary from open to compact with a wide range of dimensions; basal plant color can be either tan or colored (red or purple); grain color can vary widely depending upon the specific genotype at over seven loci that influence the appearance of the grain (Rooney and Miller, 1982). These include the color of the pericarp, the presence or absence of a testa, the thickness of the mesocarp, and the color of the endosperm.

As sorghum evolved near the equator, most sorghums are photoperiod-sensitive, with floral differentiation triggered by short days. It was only after the identification of

photoperiod insensitive sorghums that sorghum was considered a grain crop in temperate regions of the world. Given favorable environmental conditions, sorghum tillers readily and can even produce a ratoon crop, which might be considered a desirable trait depending upon the agronomic systems in which the crop is grown.

Sorghum Production

Sorghum is adapted and grown in a wide range of environments and is able to yield under stressful conditions where other cereals do not. Numerous morphological and physiological characteristics, including a deep dense root system and the ability to curl its leaves inwardly to reduce photosynthetic rate, provide sorghum a morphological advantage over other crops under drought conditions. Due to this higher stress tolerance, sorghum is grown in the semi-arid regions of the world as a staple crop. It is grown for its grain, stalk, and fiber and as forage, which can be processed to obtain sub products such as fermented foods and beverages, sugars and building materials. However, in industrialized countries, grain sorghum is grown mainly for livestock feed.

The grain yield potential of sorghum is quite high, and comparable with crops such as corn and rice. Under optimal field conditions, grain yields can reach 15 MT ha^{-1}; with good yields ranging from 7 to 9 MT ha^{-1}, when rainfall is not a limiting factor. Under average conditions, sorghum yields can vary between 3 and 4 MT ha^{-1}; and decrease to 0.3 to 1 MT ha^{-1} under drought conditions (House, 1985). According to

Unger and Baumhardt (1999) grain yields of dryland sorghum in the U.S. can be as high as 6 MT ha^{-1}.

Sorghum Improvement

The yields described in the previous section represent sixty years of continuous breeding efforts to improve the stress tolerance, yield potential and quality of sorghum. Grain sorghum yields increased 139% from 1956 to 1997, and 40% of this increase was due to the adoption of improved higher yielding and more stable hybrids (Maunder, 1999). Just seven years after the development of hybrid sorghums, yields almost doubled due to hybrids and improved agronomic inputs (Figure 1). After this period, breeding efforts focused on the introgression of yellow endosperm germplasm into established sorghums, and the utilization of male male-sterile Wheatland into hybrid pedigrees that increased grain yield 30% compared to the original hybrids (Maunder, 1999). Sorghum hybrid grain yields in the 1970s were not much higher compared to previous years, mainly due to the increased pressure of biotic stresses, such as insects, viruses, and diseases, that limited the expression of the yield potential bred into the germplasm. The scope of the breeding programs was shifted from an aggressive breeding methodology to increase potential yield, to a defensive strategy where identification followed by backcrossing of resistance genes into elite germplasm played a main role in potential yield protection. During this time, annual yield gains dropped from 2.0% to 0.9%, and even more if the comparison is made to the first 10 years of hybrids (Maunder, 1999). Although gains were reduced, breeders still made progress. This progress was made

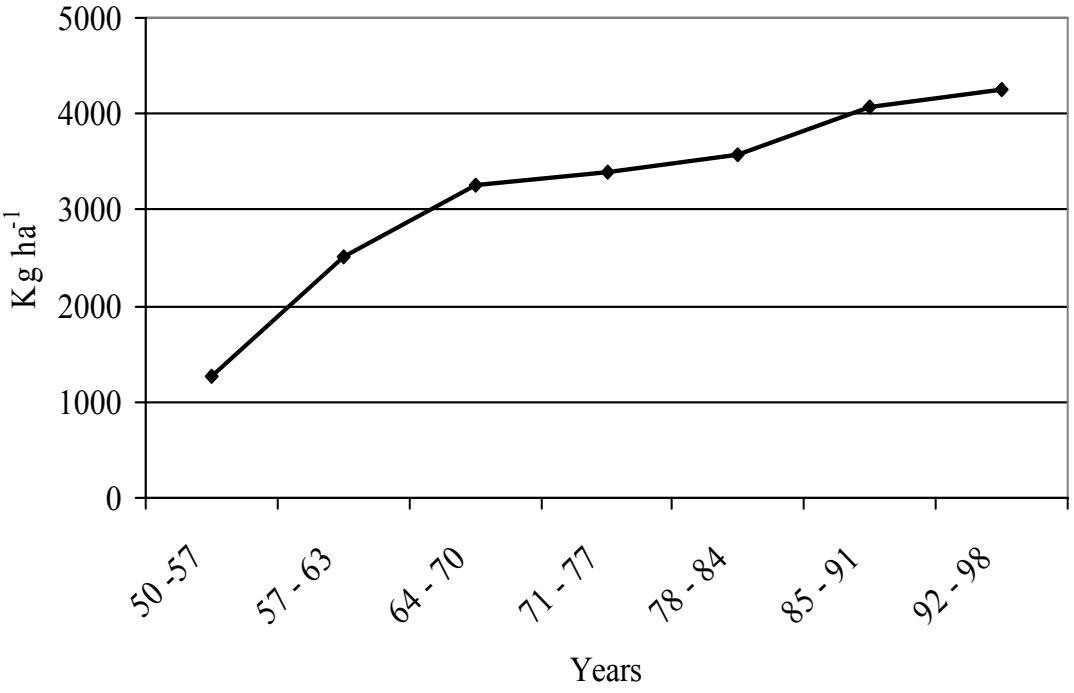

Figure 1. Sorghum yields in 7-year increment, 1950-1998. Source: Maunder, 1999.

while additional breeding emphasis was placed on stress tolerance, pest resistance, and improved quality. Breeding strategies were shifted to the identification of traits that would provide the germplasm with increased tolerance under extreme drought conditions.

Today, several factors threaten future genetic improvement of sorghum and the potential to increase grain yield. More than 80% of the sorghum in the U.S. is grown under non-irrigated conditions where water is the major limiting factor (Crasta et al.,

1999). With reduced reliance on irrigation, breeders are faced with the task of breeding new germplasm capable of producing with limited inputs, in addition to having pest resistance to protect such yield potential. However, breeding for complex quantitative traits such as improved grain yield under stressful conditions is difficult. Genetic gain depends on the ability to identify superior lines within a variable germplasm pool, but genetic variance and heritability are usually low in stress environments. This made selection more difficult in stress environments. Rosenow et al. (1983) reported that despite the major research emphasis on improving quantitative traits, such as drought resistance, progress in this regard has been slow.

Molecular Breeding

The use of molecular markers to analyze the inheritance of quantitative traits has great potential for improving genetic gain and efficiency of superior genotype selection through the studying of the genetic and phenotypic basis of complex traits (Edwards et al., 1987; Paterson et al., 1988). However, before progress can be achieved, scientists need to gather precise information about the number of loci involved in trait expression, their location along the chromosomes, and the relative contribution on trait expression of each loci (Stuber, 1992). Utilization of specific recombinant populations to construct molecular-linkage maps, allows scientists to estimate the number of loci controlling genetic variations of quantitative traits, and even identify precisely the location of such QTL in the genome, with the major goal of predicting which individuals will produce progeny showing particular genotypes (Paterson et al., 1991a; Klein et al., 2001).

Paterson et al. (1991a) reported that for a quantitative trait with low heritability, such as soluble solid in tomatoes, genotypic selection at QTL instead of phenotypic selection of the F_2 parent more accurately predicts the phenotype of the F_3 progeny, demonstrating the potential of molecular marker technology in crop improvement. Identification of candidate QTL, elucidation of epistatic and pleiotropic relationships, as well as the genetic basis of heterosis, may provide the necessary tools to allow significant advances in plant improvement and elite germplasm identification (Stuber, 1992).

Quantitative Trait Loci Mapping

QTL mapping provides information about the genetic control, location, importance, and number of genes that influence a quantitative trait, through the identification and analysis of their action, interaction and precise location in the genome. Nevertheless, the main objective in QTL mapping is to utilize the information generated in a marker assisted selection (MAS) breeding program. Construction of a well-saturated marker linkage map is the most fundamental step required for a detailed genetic study and MAS approach in any crop (Tanksley et al., 1989). Once the linkage map is constructed, associations between the marker alleles and the QTL might be found and utilized to develop improved lines or populations (Dudley, 1993). A “good” genetic marker will be one that is tightly linked to the gene(s) of interest, or even better, two closely linked flanking markers to the QTL.

Due to the high cost associated with this technology, QTL-MAS must be utilized when the trait is controlled by a few major genes that have a large environmental variance, or a large number of genes with small effects. Either way, the utilization of marker technology must be more economically feasible than scoring the trait per se.

Different types of populations may be developed for mapping purposes. The type chosen depends on the available resources, such as infrastructure, funds, and labor, as well as timeline to project completion. An F_2 population undergoes just one cycle of meiosis, has all possible combination of parental alleles, is easy to construct, can be developed quickly, is recombinant along each pair of homologous chromosomes, provides finite supply of seed tissue, and has as much as twice the information generated by a backcross individual. Backcross populations undergo just one cycle of meiosis, can be developed quickly, and are recombinant only along one of each pair of homologous chromosomes. Double haploid (DH) populations may be propagated indefinitely, present perfect homozygosity, are faster to develop than recombinant inbred lines (RIL), and present the same amount of recombination information as a backcross population. However, besides being labor intensive, this type of population may have somaclonal variation and aberrant segregation ratios due to tissue culture response.

The RIL population is among the most widely used mapping population due to its many advantages. A RIL population may be propagated indefinitely, which allows for multilocation testing, possibly decreasing error variance while increasing phenotypic variance. Also, fewer individuals are needed to detect linkage of the same magnitude as F_2's due to multiple meiotic events during population development. Precision is

increased up to two times that of F_2's, resulting in higher resolution maps. The absence of heterozygotes equalizes marker types. However, as occurs in DH populations, this characteristic precludes the estimation of dominant gene effects, which are of vital importance in heterosis. The development of RIL populations is time consuming, taking several years.

Finally, near isogenic lines (NIL) are developed by either backcrossing the progeny to one of the parents, or by selfing and selecting families segregating for the trait of interest. Either methodology will result in a population in which the locus of interest differs among individuals with the same genetic background. NIL are time consuming, and serve only to map one trait, while still needing a segregating population to assess linkage relationships.

There are several statistical methods that may be utilized to identify associations between marker alleles and QTL. These methods are divided into three categories: Single marker analysis, which considers the association between the trait and one marker locus at a time; simple interval mapping (Lander and Botstein, 1989), in which intervals formed by pairs of adjacent makers are taken as the unit of analysis and tested for the presence of single QTL by using information from flanking markers; and composite interval mapping (Zeng, 1994), a combination of interval mapping and multiple regression analysis that uses specific marker loci to control for the presence of multiple QTL linked to the interval being considered. Composite interval mapping gives more power and precision then simple interval mapping because the effects of other QTL are

not present as residual variance, and it removes the bias that would normally be caused by QTL that are linked to the position being tested.

Error control in QTL analysis is vital to define real associations between markers and QTL. As the number of markers utilized in a study increases, the probability of declaring certain effects significant when in reality there is no association also increases (Dudley, 1993). These false positives are the result of an improperly set Type 1 error control level. In contrast, accepting that there is no association between the marker(s) and the QTL, when there is a significant effect is designated as Type 2 error. Researchers are inconclusive with regard to the correct methodology to set probability levels to control for either type of error (Lander and Botstein, 1989; Zehr et al., 1992; Bubeck et al., 1993). Dividing the comparison error rate by the number of tests performed (Bonferroni approach), or by the number of chromosome arms (*ad hoc*) are two of the simplest alternatives available to adjust experiment wise error in QTL analysis. A more complex approach to adjust experiment-wise error was proposed by Churchill and Doerge (1994); a permutation test is run by shuffling the genotypic and phenotypic data while detecting the presence of QTL-marker association. This process is repeated 1,000 times while recording the highest test statistic of the association tests performed for all loci on each run. Once all the recorded test statistics are ordered, the 90, 95, 97.5, and 99^{th} percentile values are then the experiment wise levels at $\alpha = 0.1$, 0.05, 0.025, and 0.01, respectively (Basten et al., 2002). As a general rule, Lander and Botstein (1989), suggested using a log-odds ratio (LOD = $\log_{10}$ [likelihood QTL is linked to marker/likelihood there is no QTL]) between 2 and 3, to ensure a Type 1 error

rate for QTL detection of 5%. Any of these methodologies may be utilized to set the probability level when a QTL is declared as "real". However, a researcher must realize that the experiment wise probability must be a balance between the amount of false positives and negatives that may be allowed to occur. If the probability level is lowered to preclude false positives from occurring, then the probability of false negatives increases. In the end, the scope of the breeding program will dictate the proper experiment wise error level to utilize according to the risks the researchers is willing to take.

Sorghum Linkage Maps

Genetic maps based on molecular markers have several advantages over classical maps (Subudhi and Nguyen, 2000). Neutrality, abundance, and codominant nature of some type of DNA markers allow for thorough coverage of entire species genomes, addressing questions of evolution, genetic diversity, and phylogeny relevant to germplasm selection and improvement. Different types of molecular markers have been utilized to develop genetic maps in a great number of plant species of economic importance, such as tomato, maize, soybean, lettuce, wheat, pine, potato, and sugar beet, among others (Stuber, 1992).

In sorghum, several linkage maps have been developed (Subudhi and Nguyen, 2000). Hulbert et al. (1990) developed the first sorghum genome map using DNA probes that were previously mapped in the maize genome. Pereira et al. (1994)

developed a sorghum linkage map with 10 complete linkage groups using maize and sorghum probes. To increase the effectiveness of mapping information and facilitate efforts to map agronomically important traits, Tao et al. (1993), by utilizing a variety of probes, including sorghum, maize and sugarcane genomic DNA, maize and sugarcane cDNA, cereal anchor probes and eight SSR loci, reviewed and compared their sorghum map with other published maps. Subudhi and Nguyen (2000) aligned the 10 linkage sorghum groups using information generated from a RIL population, sorghum and maize probes, as well as cereal anchors from three different linkage maps (Chittenden et al., 1994; Raghab et al., 1994; Xu et al., 1994). One of the most complete sorghum genetic maps available today was published by Menz et al. (2002), who constructed a 1713 cM high-density map using 2454 AFLPs, 136 SSRs previously mapped in sorghum, and 203 cDNAs and genomic clones from rice, barley, oat, and maize.

The information generated by these genetic maps will be of vital importance for plant improvement, linking information derived by plant breeders and plant biology scientists that will allow the identification and insertion of useful agronomic genes into cultivars, positional cloning of genes, possible widening of genetic pools through comparative genomics among related and unrelated species, as well as elucidation of complex biological processes directly related to superior agronomic performance in elite germplasm (Pereira and Lee, 1995).

Quantitative Trait Loci in Sorghum

Utilization of molecular markers in germplasm improvement presents many advantages to plant breeders. They are commonly used for proprietary control of elite germplasm through molecular fingerprinting. Also, with the aid of genetic maps, researchers have found useful linkages between molecular markers and qualitative/quantitative agronomic traits. In addition to providing important information on the genetic inheritance of these traits, these linkages may be utilized in marker assisted selection programs to facilitate germplasm advancement, especially during off-season growing, pyramiding or stacking of several "resistance" genes, and introgression of "exotic" genes into elite germplasm.

Many quantitative agronomic traits have been mapped in the sorghum genome using molecular genetic tools. Among these traits are drought resistance, grain quality, yield components, pest resistance, morphological traits, and domestication-related characters, (Subudhi and Nguyen, 2000). One of the earliest works in QTL detection in sorghum was done by Lin et al. (1995) who identified six plant height QTL and three flowering QTL that accounted for 71.9% and 85.7% of the total phenotypic variation, respectively. Pereira and Lee (1995) identified four plant height QTL in regions that are orthologous to those for plant height in maize. Several QTL associated with six panicle related traits were identified by Pereira et al. (1995). These traits were panicle length, seed branch length, length of sterile portion of seed branch, peduncle number, number of seed branches per panicle, and 100-seed weight. QTL identified per trait explained as little phenotypic variation as 28% for 100-seed weight, and as much as 70% for panicle

length. Rami et al. (1998) reported two QTL explaining 67.3% of the plant height phenotypic variation observed.

Due to its wide adaptation to harsh environments, sorghum has been widely studied to identify genomic regions related to drought tolerance. Tuinstra et al. (1996) mapped six regions associated with pre-flowering stress in a RIL population; the amount of phenotypic variation explained by the genotypic data for traits associated with pre-flowering drought tolerance ranged between 14 and 43%. Post-flowering drought stress has been studied as well. Tuinstra et al. (1997), Crasta et al. (1999) and Xu et al. (2000) reported two, three, and four major QTL associated with the "stay green" trait, respectively. This trait has been recognized as a major mechanism of post-flowering drought-stress tolerance in sorghum (Rosenow et al., 1996). By comparing previous published results with their own, Kebede et al. (2001) identified three stay green QTL that were consistent across mapping populations and were developed using two different sources of the stay green trait. Also, two of these QTL have shown correspondence with drought related genomic regions in maize and rice, suggesting that these regions may have been conserved during drought tolerance evolution in these species. These regions may be targeted for high-resolution mapping to better understand the physiological mechanisms of improved drought tolerance, and eventually, cloning of stay green genes to facilitate development of drought tolerant germplasm.

Genomic regions responsible for variation in tiller number, rhizomatousness, and ratooning ability have been identified in intra and interspecific crosses of *Sorghum* (Lin et al., 1995; Hart et al., 2001). Cloning and utilization of genes in these regions may

have a direct impact on two major areas, suppression of weed propagation through plant growth regulation and improvement of forage, turf, and "ratoon" crop production (Subudhi and Nguyen, 2000). Grain quality-related traits such as dehulling yield, kernel flouriness, friability, hardness and weight, as well as amylose, protein, and lipid content were studied by Rami et al. (1998). At least one QTL was identified for each of the traits studied, with phenotypic variation explained percentages ranging from 13.7% for kernel friability, to 57.1% for kernel flouriness.

Due to the severe detrimental effect that some diseases and insect pests have on sorghum production, scientists have been trying to identify genomic regions responsible for resistance and tolerance to such pests. Progress in gene identification technology and statistical tools have allowed scientists to focus on quantitatively inherited resistance to plant diseases and insects. Klein et al. (2001) identified QTL for foliar disease and grain mold resistance in a sorghum RIL population. Five QTL affected grain-mould incidence, each accounting for 10 to 23% of the phenotypic variation observed. A QTL in linkage group (LG) I appears to be correlated to disease reaction for a series of pathogenic foliar diseases. Proximity of the plant color locus to this QTL, as well as correlations between variation in plant color and foliar disease resistance, suggests a strong relationship between such traits, as it has been long observed by sorghum breeders and pathologists (Torres-Montalvo et al., 1992). Agrama et al. (2002) identified several QTL affecting both resistance and tolerance to two greenbug (*Schizaphids graminum* Rondani) biotypes. Due to the ability of this pest to change biotypes, markers linked to the QTL

might be utilized for marker-assisted selection to effectively deploy genetic resistance genes and extend the useful life of elite germplasm.

The first reports of genomic regions associated with grain yield per se, and grain yield components (seed set and height) were done by Tuinstra et al. (1996), who measured grain yield under drought and fully irrigated conditions to test the pre-flowering drought tolerance of the germplasm. The same population was utilized by Tuinstra et al. (1997) to identify QTL for yield and their association with post-flowering drought stress QTL. Paterson et al. (1998) located nine and four QTL correlated to phenotypic variation of seed size and number, respectively. A major chromosomal region involved in "grain yield components" was identified on LG A in a *Sorghum caudatum* x *S. guinea* RIL population (Rami et al., 1998). QTL for germination rate, kernels per panicle, kernel weight per panicle, and thousand-kernel weight were detected in association with QTL for plant height, and panicle compactness and length. Presence of dwarfing genes on LG A with pleiotropic effects on morphological and productivity traits may be directly responsible for the detection of multiple QTL in this genomic region. Hart et al. (2001) mapped several QTL that control various morphological and physiological traits directly related with grain yield variation in a BTx623 x IS3620C sorghum RIL population. These QTL were located in linkage groups A, E, G, and I, explaining as much as 85.9% of the phenotypic variation of the trait, as did the QTL for panicle width.

Molecular markers linked to major QTL may be utilized to fix these regions into breeding populations in early stages of development. However, molecular breeders

should focus on identifying QTL with smaller but specific effects and fixing them in the germplasm, since their effects are more difficult to identify and fix through classical breeding.

Quantitative Trait Loci Stability

A key step in germplasm improvement is the accurate selection of superior genotypes with improved performance for at least one specific trait (Paterson et al., 1991b). Often, breeders must deal with several environmental factors that cause differential cultivar performance. This differential performance is known as the genotype x environment (GE) interaction (Fehr, 1991). The success of any breeding program will depend upon identifying the factors which influence this GE interaction and taking appropriate steps to isolate and control their effect through adequate experimental design, appropriate cultural practices, and multilocation testing. While the traditional approaches for assessing GE interaction have been successful, they are an expensive and require significant amounts of time. GE interaction assessment may benefit from the utilization of QTL mapping, since molecular markers may be utilized to perform accurate selection of genetically superior individuals from among masses of candidates, including many pretenders that were favored by environment rather than superior genetics (Paterson et al., 1991b).

QTL that show consistent expression across diverse environments are ideal candidates for MAS (Velboom and Lee, 1996). However, identification of such QTL has

been difficult. Stuber et al. (1992) reported little marker locus by environment interaction in maize, while Bubeck et al. (1993) found no consistency between environments in markers associated with QTL in the same crop. Differences in QTL detection among environments may be a function of the trait under study and the gene actions that operate upon it (Dudley, 1993). However, this may only be one of the factors that should be considered. In reality, population type and size, geographical adaptation, filial generation of evaluation, number of environments, and experimental design, are among the factors that affect the ability to detect significant associations between environment, QTL and marker loci (Beavis, 1994).

QTL analysis across environments has been widely studied in rice. Xu (2002) reported that QTL sharing frequencies between two environments varied from 9.5% for drought avoidance to 52.9% for 1000-grain weight. In average, 30% of the QTL under study were shared between both environments. Generally, QTL that explained a large portion of the phenotypic variance (flooding tolerance and paste viscosity) had the highest sharing frequency between environments. As the number of environments under study increased, the QTL sharing frequencies decreased. Xing et al. (2002) found that about 50% of the QTL associated with grain yield in rice, were influenced by environmental conditions in two consecutive years. However, the effects of such interactions were too small to be considered of great importance. Zhuang et al. (1997) concluded that QTL stability is trait dependent, as Dudley (1993) had suggested previously. QTL for grain weight per plant, 1000-grain weight, and panicle length were detected across generations and/or environments; while QTL for number of panicles per

plant, number of filled grains per plant, and spikelet fertility among others, varied considerably across different environments. In general, QTL stability was directly related to QTL LOD score and amount of phenotypic variation explained.

In sorghum, similar findings have been reported. Tuinstra et al. (1996) reported that even under differential stress conditions, staygreen QTL showed significant or near-significant associations with drought tolerance in different years. Crasta et al. (1999) and Kebede et al. (2001) also reported that major QTL associated with staygreen were consistently identified across environments. Nevertheless, Tuinstra et al. (1997) concluded that markers associated with yield, yield stability, and seed weight stability were particularly variable in different evaluation environments.

The identification of QTL expressed across environments would be a primary objective in any molecular breeding program, because they establish a set of basic genomic blocks to introgress into elite genotypes. However, the identification of environment-sensible QTL represents an opportunity to breeders. The environment-consistent QTL may be utilized to breed for "stable" cultivars and then be combined with diverse environment-sensitive QTL into a single cultivar to yield a genotype that is buffered against environment related variations (Paterson et al. 1991b).

QTL that are stable across environments may be identified by two methodologies: (1) QTL mapping data from different environments is combined and only QTL that are statistically significant on average across environments are selected. (2) QTL analyses are performed for each environment separately and environment specific QTL are identified. Then, only those QTL that are present in more than one or

two environments are declared significant. Again, the best type of methodology to utilize depends on the type and biology of the trait under study. While some traits such as staygreen in sorghum may be analyzed using data averaged across environments, traits like grain yield and yield components may have to be analyzed through environment specific analyses.

Heterosis and QTL Mapping

Because sorghum is grown as a hybrid crop, the value of a parental line must be identified through testcrossing and progeny testing. Testcrossing differentiates superior from average genotypes, as well as specific from general combiners. Depending upon the scope of the breeding program, the number of crosses per year may vary from less than a hundred to several thousand. All of these have to be tested across several environments. This process, while absolutely necessary, is labor intensive, time consuming, and utilizes a great percentage of operating budgets. Utilization of molecular marker technology would allow the identification of specific genetic factors contributing to hybrid performance or heterosis, possibly reducing or even eliminating the need for testcrossing and progeny testing.

It is well known that the success of crop hybridization relies on the correct classification of germplasm into groups that exploit heterotic patterns. Crosses between unrelated or genetically distant parental lines that belong to different heterotic groups, show greater hybrid vigor than crosses between genetically related parental lines

(Stuber, 1999). Researchers have been trying to predict increased heterosis by measuring the genetic distance computed using marker data on parental lines. However, it has been well documented that correlations between hybrid performance and molecular marker diversity of parental lines are too low to be of practical value (Dudley et al, 1991). Inadequate genome coverage, poor marker-QTL associations, misunderstanding of the dominance gene action on heterosis, are among the reasons for low correlations between lines per se marker heterozygosity and hybrid performance (Bernardo, 1992).

It is quite clear that more detailed mapping is needed before hybrid performance may be predicted based on parental line marker diversity. As mentioned before, data generated from genetic distance analysis aids in the correct classification of germplasm into heterotic groups. However, it does not provide any information with regard to specific genomic regions that may maximize heterosis. Such information must be generated through fine QTL mapping on lines per se as well as testcrosses, in order to identify not only those regions of importance for a lines per se performance, but also those that are vital for excel hybrid performance. This information may be utilized to target specific genomic regions that are directly responsible for superior hybrid performance, identifying markers linked to such regions and utilizing them for better classification of germplasm into enhanced heterotic groups.

CHAPTER III

HERITABILITY ESTIMATES FOR GRAIN YIELD AND YIELD RELATED TRAITS IN A *Sorghum bicolor* (L.) Moench RECOMBINANT INBRED RESTORER LINE POPULATION

Introduction

The success of any plant breeding program is based on the ability of the breeder to identify superior genotypes within segregating populations. However, if genetic variation is minimal, there is little possibility of genetic gain. Thus, if genetic variability is not present in the breeding populations, the researcher must identify and bring the variability to the program. Once variation is present, he or she must apply breeding techniques appropriate to the trait of interest and utilize a wide array of testing techniques and sites to maximize genetic gains.

Trait heritability estimation is a first step towards assessing the amount of genetic variation present in a breeding population. Regardless of the type of heritability estimate, heritability broadly defined, is the proportion of observable field variation that is due to genetic factors (Nyquist, 1991). Several studies have been conducted on sorghum populations to estimate trait heritabilities and phenotypic and genotypic correlations among those traits. (Liang et al., 1969; Chung and Liang et al., 1970; Lothrop et al.,

1985a; Sanchez-Gomez, 2002). Reported heritability estimates for grain yield have been somewhat variable. Discrepancies may be due to a wide range of factors including the utilization of different estimation methodologies, type of population (wide crosses), diverse environments, and generation of evaluation.

Over the past 100 years, plant breeding programs have made steady genetic gains in most major crops in the U.S. (Troyer, 1999). However, the rate of gain has been reduced in recent years (Maunder, 1999). While there are several possible reasons for the reduction, scientists are concerned that pools of genetic diversity are being narrowed by the continuous recycling of elite inbred lines. To alleviate this problem, programs to introgress exotic germplasm are used to introduce variation into elite germplasm. This approach has been successful for simply inherited traits such as pest resistance, but it has had limited success for complex polygenic traits such as grain yield. Introgression of worthless genetic blocks along with few exotic "yield" genes have limited the potential utilization of such resources for enhancing genetic gain.

An alternative approach to sustaining genetic variation in crop improvement programs is to utilize existing adapted germplasm and rely on the presence of de novo generation of genetic variation (Rasmusson and Phillips, 1997). In addition to being adapted to production systems with which the breeder is working, this germplasm may contain valuable alleles that were not included in advanced germplasm during the breeding process. This study represents an attempt to estimate the amount of genetic variability that may be exploited from the utilization of old elite lines to create new breeding populations, and to determine if any elite genes from the old germplasm are

missing in the new genetic pools. It is of crucial importance to establish if such "old" genes can be useful in current attempts to increase genetic gains, and if so, determine if these genes will still perform well in hybrid combination.

Therefore, the objectives of this research were (1) to estimate heritability on seven agronomic traits in a recombinant inbred line (RIL) population, (2) to estimate heritability on seven agronomic traits in testcross population derived from the RIL population, (3) to compare heritability estimates across populations, and (4) to estimate phenotypic and genotypic correlations among variables studied in both types of populations.

Materials and Methods

Plant Materials

A mapping population composed of 187 recombinant inbred lines (RIL) was derived from the cross of RTx430 and RTx7000 (See appendix). RTx7000 (also known as SA700, Caprock) was widely grown in the U.S. High Plains as a cultivar prior to the introduction of sorghum hybrids. It was released as a pollinator for hybrid production in 1957 and used to create several hybrids that were produced in the late 1950's and early 1960's (King et al., 1961). RTx7000 is early maturing, wide adaptation, and susceptible to most foliar pathogens. The pericarp is red and the lemmas bear short awns. RTx430

was released by the Texas Agricultural Experiment Station in 1976 (Miller, 1984). The pedigree of RTx430 is (Tx2536 x SC170-6-5-1-E2)-10-4-4-1-4-⊗. RTx430 is classified as a feterita-zera zera restorer line that is genetically a three-dwarf, with white epicarp, yellow endosperm and purple plant color. While the line per se is average for yield and quality, it has been utilized widely as a pollinator in sorghum hybrid production due to its excellent combining ability. RTx7000 and RTx430 are representative of the old and new restorer line germplasm, respectively.

The population was developed using standard plant breeding methodology. Both parental lines were hand emasculated and crossed reciprocally to obtain F_1 hybrid seed. Panicles were bagged and self pollinated to advance the population to the F_2 generation. One hundred ninety eight individual plants were randomly selected, panicles bagged, and allowed to self pollinate in the F_2 generation. One panicle from each $F_{2:3}$-derived family was randomly selected, bagged and self pollinated for generation advancement. The same process was repeated each filial generation until a $F_{5:6}$ recombinant inbred line (RIL) population was developed. At this generation, three to five panicles from each line were bagged, self pollinated, harvested and bulked for replicated agronomic evaluation of RIL per se.

Because performance in hybrid combination is more important than the line per se, a testcross hybrid population was developed as well. In a crossing block in the TAES sorghum breeding nursery, each RIL was used as a pollinator onto ATx2752. ATx2752 is a male-sterile line commonly used as a female in several commercially grown hybrids. The line has good combining ability with both RTx430 and RTx7000. BTx2752 is a

maintainer line with red epicarp, thick mesocarp and colored plant that reaches mid-anthesis in 71 days (Johnson et al., 1982). For each line, testcross hybrid seed was harvested and bulked for replicated agronomic evaluation of testcross hybrids.

Experimental Design

Both populations, the RIL per se (RIL) and testcross hybrids (TCH), were evaluated at three environments at two locations. In 1999, 198 RIL and two checks (RTx430 and RTx7000) were evaluated at Beeville (BEI99) and College Station (CSI99), Texas. Due to limited seed stocks, in 2000 only 191 RIL and five checks (RTx430, RTx7000, RTx436, ATx2752*RTx430, and ATx2752*RTx430) were evaluated at College Station (CSI00) and Beeville (BEI00), Texas. Extra checks were included to allow environmental effect comparisons between RIL and TCH, as well as with another RIL population developed using maintainer lines (Sanchez-Gomez, 2002). The BEI00 trial was lost due to high temperatures and lack of precipitation that prevented normal plant development and acceptable levels of seed set.

In separate trials, 198 testcross hybrids, six public hybrids (ATx2752, ATx623, and ATx3197 crossed to RTx430 and RTx7000), and two commercial hybrids (Dekalb-DK69 and Pioneer-8282) were evaluated at Taft (TAH00) and College Station (CSH00), Texas. In 2001, 175 TCH and checks planted at TAH00 and CSH00 were evaluated at College Station (CSH01), Texas. All experiments but TAH00 were planted at the Texas

Agricultural Experiment Station (TAES) farms at each location. TAH00 was planted at a commercial field managed by Pioneer Hi-Bred International research personnel.

BEI99 and CSI99 were planted on March 13 and 24, 1999, respectively; TAH00 was planted on February 29, 2000, while CSI00 and CSH00, were planted on May 12 and March 31, 2000, respectively; and CSH01 was planted on March 22, 2001.

Experimental units at BEI99 and CSI99 consisted of one row plots that measured 4.947 m^2 and 4.104 m^2, respectively. For experiments evaluated during 2000 and 2001, the experimental units were doubled to two row plots measuring 6.75 m^2 at Taft, and 8.208 m^2 at College Station. Each plot was replicated twice and arranged in a randomized complete block design (RCBD).

BEI99 and TAH99 were rain fed environments, and at the College Station environments, furrow irrigation was provided when needed to ensure good experiment establishment and development. Standard cultural practices, including fertilization, weed and insect control, were followed at all environments to minimize exogenous variability that would otherwise mask variability due to genetic differences of the germplasm evaluated.

Phenotypic Evaluation

Six phenotypic characters were measured in both populations (RIL and TCH) at all environments. Days to mid-anthesis (DMA) were recorded as the number of days from planting until 50% of the main panicles were at mid-anthesis. Plant height (PHE)

was measured from the ground to the tip of the main tiller; the panicle length (PLE) was recorded as the distance from the base to the tip of the main panicle; and panicle exsertion (PEX) was measured from base of the flag leaf blade to the base of the main panicle. PHE, PLE and PEX are recorded in centimeters using an average from three typical plants. Panicles per plot, including tillers, were recorded by counting the number of heads in the experimental units, which were later extrapolated to panicles per hectare (PAN). This measurement provided an estimate of the tillering ability of each genotype evaluated. Measurements for PHE, PLE, PEX and PAN were collected one week prior to harvest. Panicles were hand harvested in BEI99 and CSI99, threshed using an Almaco® Large Plot Thresher, and grain weighed to estimate grain yield. Plots in the other environments were combine harvested using a Massey Ferguson XP8 research plot combine. Plot grain yield data from all environments was converted to metric tons of grain per hectare (MT ha^{-1}) adjusting the weights to 13% moisture (GYL).

Due to variability in plot plant stands among and within entries, and to study the correlation of such variability with productivity, GYL and PAN variable were combined into an artificial variable designated as panicle weight (PWE). This variable was calculated by dividing the weight of the harvested grain per plot, by the number of panicles counted in that specific plot.

Statistical Analyses

Once RIL and TCH data from all environments were collected, data from 186 RIL and 182 TCH was utilized to performed all statistical analyses. Several RIL and testcross hybrids were eliminated from the data set because of inconsistencies in the observed plots and to establish consistency between the phenotypic and genotypic analyses on the germplasm.

For both, lines per se and hybrids, two different analyses of variance were calculated. For the first, the RIL per se and the parental lines were utilized. This was necessary to determine if any differences observed between the parental and the recombinant lines were statistically significant. For the second analysis, data from parental lines was excluded to obtain statistical parameters that apply only to the segregant population.

Individual environment and combined analyses for the RIL and TCH populations were analyzed following a randomized complete block design (Tables 1 and 2). For each type of population, individual analyses of variance were generated using two different statistical procedures, the General Linear Model (GLM) and the Mixed Model (MIXED) procedures included in SAS 8.0®. GLM uses the method of least squares to fit general linear models on the means, while MIXED fits the model based on means, variances and covariances. Variances estimated by both types of procedures may differ due to the inherent nature of the method of estimation. Thus, variances were obtained using both types of procedures and further analyzed to establish which method was the most efficient at assessing variability observed in both types of populations.

The linear model utilized for individual analyses was as follows:

$y_{ij} = \mu + \rho_i + \alpha_j + \varepsilon_{ij}$

where

μ is the population mean;

α_i is the effect due to the *i*-th genotype;

ρ_j is the effect due to the *j*-th replicate;

ε_{ij} is the environmental effect associated with the *ij*-th individual observation.

For analyses of variance based on GLM and MIXED, both replications and genotypes were assumed as random effect components. Variance component estimation and F-tests between mean squares were performed based on the expected means squares (Table 1) obtained using the random option in the GLM procedure. The random option provides variance component coefficients based on harmonic means due to missing data (Tables 6, 8, 9, 11, 12, 13 and 15). The mixed model routine readily provided the variance component estimates for all random factors included in the model.

Adjusted means were obtained using the least squares means (lsmeans) option in both procedures. However, MIXED allows only the estimation of lsmeans on fixed effect factors. Thus, for the purpose of means adjustment, analyses were rerun setting genotypes as a fixed factor.

Efficiency of the MIXED analyses against the GLM analyses was assessed by calculating and comparing the standard error of the difference of the least squares means obtained by each procedure. The efficiency was defined as the squared ratio of the standard error of the difference for the GLM-lsmeans and the MIXED-lsmeans. The formula utilized was as follows:

$$Efficiency = \left(\frac{S.E.D. - GLM : LSmeans}{S.E.D. - MIXED : LSmeans} \right)^2$$

Table 1. Expected mean squares for the individual environment analysis of variance.

Source	df†	Mean Squares	Expected Mean Squares‡
Replication	r	MS_R	$\sigma_\varepsilon^2 + g'\sigma_R^2$
Genotypes	g	MS_G	$\sigma_\varepsilon^2 + r'\sigma_G^2$
Exp. Error	(r-1)(g-1)	MS_ε	σ_ε^2
Total	rg-1		

† Varied depending upon the number of missing observations at each environment.
‡ *g'* and *r'* denote harmonic means for genotypes and replications, respectively.

In order to combine data from individual environments, a Bartlett's test for heterogeneity of error variances (Steel and Torrie, 1980) was performed. Results indicated that the error variances across environments were heterogeneous. Data

transformation failed to normalize variances; thus, combined analyses of variance were computed on untransformed data.

As in the individual analyses, the combined analyses were performed using the GLM and MIXED procedures for comparison purposes. Analyses of variance were performed and variance components estimated assuming an all random factor model. Adjusted means were obtained using the lsmeans option in the GLM and MIXED procedures included in SAS® 8.0. However, as in the individual analyses, for the MIXED procedure, genotypes were set as fixed in order to obtain adjusted means. Based on the random - GLM output, F-testing was performed using the appropriate error term according to the expected mean squares (Table 2).

Harmonic means, obtained using the random option in GLM, were utilized to calculate variance estimates for the different factors included in the model. As mentioned before, MIXED output readily provided the different variance estimates without any further calculation.

The linear model utilized for the combined analyses was as follows:

$$y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \rho(\beta)_{jk} + \varepsilon_{ijk}$$

where

μ is the population mean;

α_i is the effect due to the *i*-th genotype;

β_j is the effect due to the *j*-th environment;

$\alpha\beta_{ij}$ is the effect due to the interaction of the *i*-th genotype with the *j*-th environment;

$\rho(\beta)_{jk}$ is the effect due to the *k*-th replicate within the *j*-th environment;

ε_{ijk} is the error associated with the *ij*-th individual observation.

Table 2. Expected mean squares for the combined analysis of variance across environments.

Source	df†	Mean Squares	Expected Mean Squares‡
Replication (Environment)	e(r-1)	$MS_{R(E)}$	$\sigma_\varepsilon^2 + g'\sigma_{R(E)}^2$
Environment	e-1	MS_E	$\sigma_\varepsilon^2 + g'\sigma_{R(E)}^2 + r'\sigma_{GE}^2 + r'g\sigma_E^2$
Genotypes	g-1	MS_G	$\sigma_\varepsilon^2 + r'\sigma_{GE}^2 + r'e'\sigma_G^2$
Genotype x Environment	(g-1)(e-1)	MS_{GE}	$\sigma_\varepsilon^2 + r'\sigma_{GE}^2$
Error	e(g-1)(r-1)	MS_ε	σ_ε^2
Total	egr-1		

† Varied depending upon the number of missing observations.
‡ *g'*, *r'* and *e'* denote harmonic means for genotypes, replications and environments, respectively.

For each phenotypic trait, overall means and their respective standard errors were calculated for the RIL and the parental lines to determine if there were any significant differences among them. Phenotypic correlations among the seven traits were calculated

using adjusted means obtained through the least squares means procedures included in the statistical software previously mentioned.

Broad Sense Heritability Estimation

Broad sense heritability (*H*) for the different traits in both populations was estimated using two different methodologies. In the first method, information from the GLM analyses was utilized to calculate the heritability estimates as the ratio between the genotypic and the phenotypic variances of the RIL. The estimation of *H* for the different traits by individual environment was as follows:

$$H = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_\varepsilon^2 / r'}$$

where

σ_G^2 is the genotypic variance;

σ_ε^2 is the error variance;

r' harmonic mean of replications.

Broad sense heritability estimates for the combined analyses across environments were calculated as follows:

$$H = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GE}^2 / r' + \sigma_\varepsilon^2 / r'e'}$$

where

σ_G^2 is the genotypic variance;

σ_{GE}^2 is the genotype by environment interaction variance;

σ_e^2 is the error variance;

r' is the harmonic mean of replications;

e' is the harmonic mean of environments.

Precision of the heritability estimates was assessed by calculating the lower (1 - 0.95) and upper (1 – 0.05) confidence limits as reported by Knapp et al. (1985).

In the second method, broad sense heritabilities for the different traits within one environment and across environments were estimated using the MIXED procedure included in SAS® 8.0. The program utilized was provided by Dr. James Holland (Holland et al., 2003) and is freely available on the worldwide web (http://www4.ncsu.edu/~jholland/homepage.htm). The MIXED procedure reports broad sense heritability on plot basis and genotypic mean basis and their respective standard

errors. As was the case in adjusted means calculations, comparisons between GLM and MIXED heritability estimates were performed to establish if any differences existed between them, and the extent of such differences.

Phenotypic and Genotypic Correlation

Phenotypic and genotypic correlation coefficients were estimated among the seven traits at individual environments and across all environments where the populations were evaluated.

Coefficients were estimated using a multivariate restricted maximum likelihood estimation (Holland, 2002). The estimation was done using the MIXED procedure included in SAS® 8.0. The program code is available on the worldwide web (http://www4.ncsu.edu/~jholland/homepage.htm). The MIXED procedure outputs in a matrix format the variances and covariances needed to calculate the genotypic correlation coefficient (r_G), the phenotypic correlation coefficient (r_P) and their respective standard errors between traits. The calculation of r_G was as follows:

$$r_G = \frac{Cov_G}{\sqrt{\sigma^2_{GX}\sigma^2_{GY}}}$$

where

Cov_G is the genotypic covariance between traits x and y;

σ^2_{GX} is the genotypic variance of trait x;

σ^2_{GY} is the genotypic variance of trait y.

Phenotypic correlation was calculated as r_G. However, instead of using genotypic covariances and variances, phenotypic estimates were utilized.

Results and Discussion

GLM versus MIXED

Efficiency Analyses. In general, the GLM analyses provided better estimators than the MIXED model since all efficiency ratios were equal or lower than "1" (Table 3). In CSI99, efficiency ratios ranged from 0.944 for PHE and PEX, to 0.952 for DMA, while in BEI99 efficiency ratios for all traits evaluated were approximately 0.93. Efficiency of MIXED was much lower in CSI00 when compared to the other environments where the RIL population was evaluated since all traits, with the exception of PLE, had ratios lower than 0.90. For the TCH population, GLM was more efficient in adjusting the means for variability than the MIXED procedure in two of the environments where the population was evaluated.

Analyses of CSH00 and CSH01 environments revealed that efficiency ratios for all traits ranged between 0.962 for DMA in CSH00 to 0.986 for PWE in CSH01. In TAH00 the MIXED procedure was as efficient as the GLM for all traits except GYL

where the MIXED procedure had only a 0.975 efficiency ratio when compared to the GLM.

Table 3. Efficiency of MIXED compared to GLM for seven traits evaluated in the RIL and TCH populations at different environments.

	RIL			TCH		
Trait	CSI99	BEI99	CSI00	CSH00	TAH00	CSH01
PWE	0.948	0.934	0.883	0.976	1.000	0.986
GYL	0.948	0.934	0.891	0.976	0.975	0.982
PAN	0.948	0.934	0.895	0.976	.	0.995
DMA	0.952	0.930	0.882	0.962	1.000	0.964
PHE	0.944	0.934	0.895	0.976	1.000	0.994
PEX	0.944	0.934	0.895	0.976	1.000	0.984
PLE	0.949	0.930	0.901	0.976	.	0.984

Variances. The MIXED model statistical analysis has been widely publicized as a better method for estimating variances from unbalanced data, which is the case for all trait data analyzed in this study. However, the differences observed for genetic, genotype by environments, and residual variances between the GLM and MIXED model were not as great as expected.

The GLM and MIXED models differed in their estimates of genetic, genotype by environment and error variances by an average of 5.56%, 10.86% and 1.36%, respectively (Table 4). Estimations by the MIXED procedure were somewhat larger

Table 4. Comparisons of the σ^2_G, σ^2_{GE} and σ^2_ε from the combined analyses of the RIL and TCH using the GLM and MIXED statistical procedures.

		σ^2_G		DIFF‡	σ^2_{GE}		DIFF‡	σ^2_ε		DIFF‡
Type†	Trait	GLM	MIXED	(%)	GLM	MIXED	(%)	GLM	MIXED	(%)
RIL	DMA	6.27	6.97	11.03	1.76	1.76	-0.06	4.38	4.44	1.35
RIL	PWE	26.68	26.82	0.55	18.61	18.25	-1.97	65.85	66.19	0.52
RIL	PEX	12.11	12.69	4.71	2.62	2.61	-0.47	12.75	12.78	0.26
RIL	PLE	4.31	4.24	-1.62	0.75	0.78	3.40	5.76	5.74	-0.39
RIL	PAN	4.36E8	4.74E8	8.68	3.46E8	3.41E8	-1.47	6.55E8	6.66E8	1.74
RIL	PHE	123.55	125.18	1.32	44.99	44.14	-1.89	90.70	91.50	0.88
RIL	GYL	0.33	0.33	0.18	0.46	0.46	-1.19	0.54	0.55	2.84
TCH	DMA	2.06	2.06	0.39	0.23	0.23	1.65	2.44	2.44	-0.05
TCH	PWE	6.91	6.14	-11.15	12.11	12.30	1.59	52.78	53.16	0.71
TCH	PEX	3.15	3.13	-0.71	1.86	1.85	-0.44	15.91	15.92	0.04
TCH	PLE	1.30	1.42	9.23	0.13	0.00	.	6.87	6.96	1.31
TCH	PAN	1.22E8	9.43E7	-22.61	1.74E8	2.13E8	22.77	7.61E8	7.27E8	-4.47
TCH	PHE	40.42	41.54	2.76	24.61	21.60	-12.22	68.74	71.77	4.41
TCH	GYL	0.17	0.16	-3.02	0.13	0.14	2.92	0.80	0.80	0.19

† Based on combined analyses of RIL and TCH populations.
‡ Calculated as (MIXED – GLM)/GLM x 100.

when genetic and residual variability were assessed. MIXED genetic variability was 11% larger for RIL-DMA, and 23% smaller for TCH-PAN when compared to the GLM genotypic variance estimates for the same traits. For the genotype by environment variance, the greatest contrast was observed in TCH-PLE because MIXED did not estimate variance, and GLM estimated a 0.13 variance. With the exception of TCH-PAN and TCH-PHE, percentage differences between both types of estimators were lower than 3%, which basically allows for the utilization of either type of analysis to estimate this type of variation in both types of populations.

The fact that genetic and genotype by environment variances for many traits are greater when generated by the MIXED model is quite logical, since this model theoretically minimizes the error, thus allowing for a better estimation of other sources of variability. In the current study, residual variances estimated by MIXED were usually larger than those estimated by GLM (Table 4). Nevertheless, the differences were minimal and not considered to be of great importance.

Heritability Estimates. With exception of one trait (PAN), variation in the difference between heritability estimates calculated using both types of statistical models ranged between 0 and 0.03 (Table 5). The single trait that exceeded this range was PAN in the TCH, where a difference of approximately 20% was present between the two estimates. However, no differences in estimates were observed for DMA in the testcrosses. Heritability estimates in the RIL were usually larger when estimated by GLM, but in the TCH, estimates were larger when calculated using MIXED. However,

Table 5. Comparison of the broad sense heritability estimates (*H*) from the combined analyses of the RIL and TCH populations using the GLM and MIXED statistical procedures.

Type†	Trait	*H*		DIFF. (%)
		GLM	MIXED	
RIL	DMA	0.82	0.83	1.57
RIL	PWE	0.59	0.60	0.21
RIL	PEX	0.79	0.80	0.87
RIL	PLE	0.77	0.76	-0.52
RIL	PAN	0.65	0.67	2.62
RIL	PHE	0.80	0.80	0.24
RIL	GYL	0.57	0.57	-0.39
TCH	DMA	0.80	0.80	0.00
TCH	PWE	0.34	0.31	-8.51
TCH	PEX	0.48	0.48	-0.45
TCH	PLE	0.41	0.44	6.73
TCH	PAN	0.29	0.23	-20.45
TCH	PHE	0.67	0.68	1.59
TCH	GYL	0.47	0.46	-2.24

† Based on combined analyses of RIL and TCH populations.

Pearson's correlation coefficient of 0.995 between GLM and MIXED heritabilities suggests that any differences between the two methods were of minimal practical significance.

Adjusted Means. The objective of any statistical analysis should be to produce the best means, adjusted when necessary for sources of variation that are statistically, but moreover, agronomically significant. The adjusted means produced by each type of

analyses were correlated to each other, and they were correlated to arithmetic means at each individual environment to determine the degree of adjustment performed by each type of analysis. Arithmetic means were obtained using the MEANS procedure in SAS® 8.0.

Even though correlation coefficients among the three different types of means were quite high (> 0.91), means adjusted by GLM were generally more consistent with the arithmetic means of the different traits across both populations and environments (data not shown). Closer examination of the data revealed that whenever missing data was observed, GLM did not estimate a value for such an entry, while MIXED readily produced an estimate for the missing value. It is crucial to use care when an algorithm is used to compute a new estimate when observations are few for such computation. Often these estimates are inconsistent with field data. Correlations were less consistent when adjusted means from the combined analyses were compared. This is logical as more sources of variation were utilized in the adjustment of the means. In addition, since there were few observations in each environment, observations from contrasting environments were utilized by the statistical procedure to adjust the data. Because of the significant variation among environments, these estimates were subject to larger adjustments to any prediction of a missing value.

Based on the data presented herein, GLM was a better model for mean adjustment because predicted values for missing observations were not over- or underestimated, and in some cases, not estimated. It is better not to have a predicted value that does not have any relationship to what would be a true field observation, than

to predict a value that may cloud any further analysis where such means are utilized. For all further analyses in this dissertation, GLM adjusted means were utilized.

Trait Analyses

Grain Yield. Significant differences ($P < 0.05$) were observed among environments where RIL were evaluated (Table 6). As expected, higher grain yields were observed in College Station because these environments were irrigated (Table 7). Yields in Beeville were substantially lower due to periods of drought during the growing season. In the TCH, grain yields were 5.40, 5.54, and 4.33 MT ha^{-1} in CSH00, TAH00, and CSH01, respectively (Table 7). Due to timely rains in the TAH00 environment and irrigation in CSH00 and CSH01, yields observed in TCH were more consistent across environments and no significant differences among environments were observed at the 0.05 level. Although no statistical comparisons could be done between RIL and TCH, TCH had higher grain yield means than the RIL (Table 7). While a significant ($P = 0.04$) Pearson's correlation was detected between overall grain yield RIL and TCH means, this coefficient is too low (0.17) to be of any practical importance. Thus, it can be concluded that there is no relationship between the performance of the lines per se and their testcrosses. This was expected since TCH exploit heterosis to achieve higher yields, relying on dominance and additive gene actions and their epistatic relationships, while RIL rely only on additive and epistatic gene action. Sanchez-Gomez (2002) reported similar results when yielding performance of maintainer lines was compared to their testcrosses.

Table 6. Analysis of variance for grain yield (GYL) at individual and combined environments for RIL and TCH.

Env	Source	df	M.S.	Variance Component Coefficients $\sigma_R^2/\sigma_{R(E)}^2$	σ_G^2	σ_E^2	σ_{GE}^2
CSI99	R	1	21.25**	174.00	.	.	.
	G	184	2.54**	.	1.94	.	.
	Error	173	0.77				
BEI99	R	1	1.81*	169.00	.	.	.
	G	182	0.65**	.	1.92	.	.
	Error	168	0.23				
CSI00	R	1	12.47**	158.00	.	.	.
	G	181	2.88**	.	1.87	.	.
	Error	157	0.61				
Combined RIL	R(E)	3	11.85**	167.00	.	.	.
	G	185	3.25**	.	5.53	.	1.87
	E	2	376.46*	166.88	.	333.76	1.83
	GxE	362	1.41**	.	.	.	1.89
	Error	498	0.54				
CSH00	R	1	121.12**	177.00	.	.	.
	G	181	1.54**	.	1.97	.	.
	Error	176	0.80				
TAH00	R	1	5.68*	182.00	.	.	.
	G	181	1.50**	.	2.00	.	.
	Error	181	0.95				
CSH01	R	1	1.01	163.00	.	.	.
	G	166	1.09**	.	1.98	.	.
	Error						
Combined TCH	R(E)	3	42.60**	174.00	.	.	.
	G	181	2.02**	.	5.76	.	1.98
	E	2	153.33	171.54	.	343.08	1.97
	GxE	347	1.06*	.	.	.	1.98
	Error						

** Significantly different from zero at the 0.0001 probability level.
* Significantly different from zero at the 0.05 probability level.

Table 7. Grain yield (GYL) and plant height (PHE) estimated parameters for the RIL and TCH populations by individual and combined environments.

Trait†	Environment	Mean‡ ± S.E.	L.S.D. (0.05)	C.V. (%)	σ_G^2 ± S.E.	σ_P^2	σ_E^2	σ_{GE}^2	σ_ε^2	H	H C.I.
GYL	CSI99	3.24[a] ± 0.63	1.75	26.98	0.91 ± 0.14	1.31	.	.	0.77	0.70	(0.61,0.76)
GYL	BEI99	1.52[b] ± 0.34	0.96	31.30	0.22 ± 0.04	0.34	.	.	0.23	0.65	(0.55,0.73)
GYL	CSI00	3.46[a] ± 0.57	1.60	22.66	1.21 ± 0.17	1.54	.	.	0.61	0.79	(0.72,0.83)
GYL	Combined	2.74 ± 0.50	0.80	26.76	0.33 ± 0.01	0.59	1.09	0.46	0.54	0.57	(0.47,0.65)
GYL	CSH00	5.40[a] ± 0.64	1.77	16.52	0.38 ± 0.08	0.78	.	.	0.80	0.48	(0.34,0.60)
GYL	TAH00	5.54[a] ± 0.69	1.92	17.58	0.28 ± 0.08	0.75	.	.	0.95	0.37	(0.19,0.51)
GYL	CSH01	4.33[a] ± 0.57	1.58	18.37	0.23 ± 0.06	0.55	.	.	0.63	0.42	(0.25,0.55)
GYL	Combined	5.11 ± 0.43	0.42	17.47	0.17 ± 0.004	0.35	0.32	0.13	0.80	0.47	(0.35,0.58)
PHE	CSI99	125.53[a] ± 7.62	21.27	8.44	233.60 ± 31.27	291.68	.	.	112.36	0.80	(0.74,0.84)
PHE	BEI99	96.22[c] ± 5.70	15.92	8.22	82.77 ± 12.50	115.28	.	.	62.52	0.72	(0.64,0.78)
PHE	CSI00	116.27[b] ± 7.20	20.11	8.47	192.39 ± 27.31	244.23	.	.	97.09	0.79	(0.73,0.84)
PHE	Combined	112.71 ± 5.64	7.93	8.45	123.55 ± 2.52	155.16	233.69	44.99	90.70	0.80	(0.75,0.83)
PHE	CSH00	123.87[a] ± 5.80	16.18	6.57	105.98 ± 14.79	139.57	.	.	66.26	0.76	(0.69,0.81)
PHE	TAH00	115.07[a] ± 6.61	18.44	8.12	31.91 ± 7.90	75.60	.	.	87.38	0.42	(0.26,0.55)
PHE	CSH01	133.06[a] ± 5.06	14.13	5.35	56.72 ± 9.06	82.30	.	.	50.71	0.69	(0.60,0.76)
PHE	Combined	123.72 ± 4.51	5.74	6.70	40.42 ± 0.82	60.75	69.75	24.61	68.74	0.67	(0.59,0.73)

† Mean values for GYL and PHE in MT ha^{-1} and cm, respectively.

‡ Environment means followed by the same letter are not significantly different from each other at the 0.05 level (L.S.D.).

When a recombinant inbred line population has not undergone breeding selection, it is highly probable that statistical differences will be found among the progeny. In the current study, highly significant differences ($P < 0.0001$) were observed among RIL at all individual environments and combined across environments (Table 6). However, significant differences were not observed between the parental lines in CSI99 and BEI99. In CSI00 and in the combined analysis, RTx7000 yielded higher than RTx430 (Data not shown). Six RIL yielded significantly ($P < 0.05$) more than the highest yielding parental lines in CSI99. GYL of these lines varied between 5.52 and 6.21 MT ha^{-1}, compared to 3.73 MT ha^{-1} averaged by RTx430. In BEI99, R162, R98, and R42 averaged 3.04, 2.98, and 2.69 MT ha^{-1}, which were statistically higher than yields observed in both parental lines. In CSI00, no RIL outyielded RTx7000. In the combined analysis, R108 yielded 4.45 MT ha^{-1}, which was 24% and 78% more grain yield than that produced by RTx7000 and RTx430, respectively.

Significant differences were detected among TCH ($P < 0.0001$) and between parental testcrosses ($P < 0.05$) at CSH00, CSH01 and combined analysis. Although no differences were detected between parental testcrosses in TAH00, significant differences were detected among TCH (Table 6). ATx2752 x RTx430 consistently yielded more than ATx2752 x RTx7000, with yields that averaged 56% more across all environments. Even though at least one testcross at each environment had a grain yield greater than the RTx430 testcross, such differences were not statistically significant ($P = 0.05$). When data was combined across environments, the RTx430 testcross outyielded the second highest yielding TCH by 0.30 MT ha^{-1}. Among the combined RIL and TCH means that

were not statistically lower than the highest yielding parent or parental testcross, R113 and R23 were present in both groups. These observations may have no genetic base, since the other RIL (R68) produced a high yielding testcross but were very low yielding in line per se trials.

Heritability estimates for GYL in the RIL were consistently higher than in the TCH at individual environments and combined analysis (Table 7). *H* ranged from 0.65 to 0.79 in the RIL, while in the TCH varied from 0.37 to 0.48. Overall heritability estimates were 0.57 and 0.47 for RIL and TCH, respectively. Higher residual variance observed in the combined TCH analysis may be responsible for the lower calculated estimate. However, based on the confidence intervals for both estimates, it is possible that if experiments were repeated, such difference between both estimates may not be as great or even detected. Even though the RIL estimates by individual environment are in agreement with those reported by Liang et al. (1969) and Chung and Liang (1970), the combined estimate is lower. It may be possible that the environments where the RIL were evaluated are more contrasting than those utilized by other scientists. Contrasting environments may tend to result in higher genotype by environment variance, increasing the phenotypic variance thus resulting in lower heritability.

Plant Height. Quinby (1975) described four genetic loci (designated $Dw_{1\text{-}4}$) that control large variations in height. While these four loci are not segregating in the current population, significant variation was detected in plant height among RIL. This suggests the presence of additional genetic loci that modify plant height within a dwarf class. Differences in plant height were detected among environments, with greater heights

observed in the College Station trials than Beeville (Tables 7). Poor growing conditions negatively affected the normal development of the genotypes in Beeville, preventing them from achieving higher growth rates. Plant height variation in TCH across environments was not large enough to detect differences (Table 7).

Significant differences were detected among RIL and TCH when evaluated by individual and combined environments (Table 8). RTx7000 and its testcross were consistently taller than RTx430 and its respective testcross. However, only the height difference between parental lines per se was statistically important ($P < 0.05$). Several RIL at each environment were statistically taller and smaller than RTx7000 and RTx430, respectively. Among these lines, several genotypes were identified consistently as the tallest or shorter (Data not shown). A similar trend was observed in TCH with the exception of CSH01 where few hybrids were inconsistent with their overall height across environments. In 2001, the vegetative growth stage was almost 11 days shorter than in 2000. This might have had a negative impact on the potential height that could have been reached by some of the hybrids.

A significant ($P < 0.001$) positive correlation was detected between the height of the RIL and their testcross ($r = 0.53$). As suggested by Dalton (1967), this correlation could be quite useful if a genetic correlation is detected between GYL and HEI within and across populations.

Table 8. Analysis of variance for plant height (PHE) at individual and combined environments for RIL and TCH.

Env	Source	df	M.S.	Variance Component Coefficients $\sigma_R^2/\sigma_{R(E)}^2$	σ_G^2	σ_E^2	σ_{GE}^2
CSI99	R	1	144.40	173.00	.	.	.
	G	184	564.33**	.	1.93	.	.
	Error	172	112.36				
BEI99	R	1	131.49	169.00	.	.	.
	G	182	221.70**	.	1.92	.	.
	Error	168	62.52				
CSI00	R	1	650.05*	159.00	.	.	.
	G	181	457.41**	.	1.87	.	.
	Error	158	97.09				
Combined	R(E)	3	308.65*	167.00	.	.	.
RIL	G	185	859.24**	.	5.53	.	1.87
	E	2	78398.55**	166.90	.	333.80	1.83
	GxE	362	175.90**	.	.	.	1.89
	Error	498	90.70				
CSH00	R	1	8035.27**	177.0	.	.	.
	G	181	275.30**	.	1.97	.	.
	Error	176	66.26				
TAH00	R	1	0.54	182.00	.	.	.
	G	181	151.21**	.	2.00	.	.
	Error	181	87.38				
CSH01	R	1	8.67	164.00	.	.	.
	G	166	163.11**	.	1.98	.	.
	Error	163	50.71				
Combined	R(E)	3	2681.49**	174.33	.	.	.
TCH	G	181	351.06**	.	5.78	.	1.98
	E	2	26704.99	171.87	.	343.73	1.97
	GxE	347	117.52**	.	.	.	1.98
	Error	520	68.74				

** Significantly different from zero at the 0.0001 probability level.
* Significantly different from zero at the 0.05 probability level.

Reported heritability estimates for plant height have ranged between 0.75 and 0.97 (Liang et al., 1969; Chung and Liang, 1970; Sanchez-Gomez, 2002). RIL have an overall *H* of 0.80 (Table 7), which is quite high and consistent with what is expected from an oligogenic trait. However, TCH heritability for PHE (0.67) is below the reported estimates. A large residual variance and small genetic variance observed in TAH00 compared to the other two TCH environments, had a negative effect on the overall *H* estimate. Due to problems during planting, several of the experimental units were actually one row plots. Lack of competition for water, light, and nutrients from the neighboring row, might have allowed entries in one repetition to outgrow their counterparts in the second repetition, resulting in an inflated experimental error. This inflated experimental error might have had a detrimental effect on the estimation of heritability for PHE.

Days to Mid-anthesis. For both the RIL and TCH populations, the relative maturity differed greatly among environments and genotypes (Table 9). RIL were significantly earlier in CSI00, taking them approximately 58 days to reach mid-anthesis (Table 10). Since this trial was replanted due to hail damage, this earliness is due to higher temperatures during the vegetative growth of the lines. No differences were detected between CSI99 and BEI99, since RIL averaged 70 and 72 days to reach mid-anthesis at each environment.

Across environments, RIL averaged 66 days to mid-anthesis, while TCH were later, reaching mid-anthesis in 74 days. CSH00, TAH00, and CSH01 averaged 83, 68 and 72 days, respectively, with these differences being statistically significant ($P < 0.05$).

Table 9. Analysis of variance for DMA at individual and combined environments for RIL and TCH.

Env	Source	df	M.S.	Variance Component Coefficients $\sigma_R^2/\sigma_{R(E)}^2$	σ_G^2	σ_E^2	σ_{GE}^2
CSI99	R	1	70.83**	174.00	.	.	.
	G	183	25.54**	.	1.95	.	.
	Error	173	4.07				
BEI99	R	1	131.25**	168.00	.	.	.
	G	182	25.17**	.	1.92	.	.
	Error	167	6.65				
CSI00	R	1	8.78	154.00	.	.	.
	G	179	9.93**	.	1.85	.	.
	Error	153	2.26				
Combined	R(E)	3	70.29**	165.33	.	.	.
RIL	G	185	42.12**	.	5.49	.	1.87
	E	2	17884.33**	164.77	.	329.53	1.83
	GxE	359	7.71*	.	.	.	1.89
	Error	493	4.38				
CSH00	R	1	158.69**	174.00	.	.	.
	G	181	9.58**	.	1.96	.	.
	Error	173	3.82				
TAH00	R	1	0.46	182.00	.	.	.
	G	181	5.45**	.	2.00	.	.
	Error	181	1.72				
CSH01	R	1	45.00**	160.00	.	.	.
	G	166	5.60**	.	1.96	.	.
	Error	159	1.77				
Combined	R(E)	3	68.08**	172.00	.	.	.
TCH	G	181	14.65**	.	5.72	.	1.96
	E	2	20386.47**	169.63	.	339.26	1.94
	GxE	347	2.89*	.	.	.	1.97
	Error	513	2.44				

** Significantly different from zero at the 0.0001 probability level.
* Significantly different from zero at the 0.05 probability level.

Lateness observed in CSH00 was due to a combined effect of the hail damage and cooler temperatures during the early phases of development that greatly reduced the growth rate of the TCH.

Pearson's correlation coefficient between RIL and TCH for DMA was 0.50 ($P < 0.001$). This correlation suggests that an early RIL will produce an early hybrid when testcrossed with ATx2752. However, this trend is not always consistent as examples of early RIL producing late hybrids were observed (Data not shown). This may change if another tester that differs from ATx2752 in the number of "flowering" dominant/recessive loci is utilized.

Since RTx430 was just one day later than RTx7000, there were no statistical differences in DMA between parental lines. Eighty-two and ten RIL were significantly earlier and later than the parental lines, respectively. R172 required approximately 57 days to reach mid-anthesis, while the R63 averaged 73 days to anthesis. In BEI99 and CSI00 several lines were earlier than the parental lines, but none were statistically ($P > 0.05$) later. While no differences ($P > 0.05$) were detected between parental testcrosses, TCH analyses identified one testcross that was later than the parental testcrosses and several that were earlier (Data not shown).

A significant genotype by environment interaction ($P < 0.05$) was detected in both populations, but in general, genotypes were consistent in ranking for DMA. Genotype rankings in both populations are agreement with the ANOVA results, since several RIL and TCH that were categorized as early or late were so across environments. Because DMA is an oligogenic trait, it should be less affected by shifts in environmental

conditions than would a polygenic trait like GYL. It is more likely that the populations as a whole may be affected with a decrease or increase in the amount of time needed to reach anthesis.

While the TCH genetic, genotype by environment, and error variances were arithmetically smaller than the RIL variances, the TCH environmental variance was proportionally larger than the RIL environmental variance for DMA (Table 10). Inflated environmental variance is a direct result of hail damage that delayed the onset of anthesis on TCH at CSH00, which affected DMA distribution of TCH across all environments. However, since σ_E^2 is not considered to estimate DMA heritability and other variances are more consistent across populations, combined *H* for RIL and TCH were 0.82 and 0.80, respectively (Table 10); which was expected since DMA in these population is controlled by modifier genes only. In general, heritability estimates at individual environments were more consistent in the RIL than in TCH (Table 10).

TCH heritabilities at individual environments were up to 20% (CSH00) lower than the combined estimate. Closer examination of the data reveals two possible reasons for the reduction. First, in TAH00 and CSH01, lower genetic variability estimates are likely responsible for the lower estimates. Second, in CSH00, a large error variance is the cause of the reduction. Because of these factors, for DMA the combined analysis actually strengthens the results since the phenotypic variance was proportionally decreased due to a much smaller error variance estimate.

Table 10. Days to mid-anthesis (DMA) and tillering ability (PAN) estimated parameters for the RIL and TCH populations by individual and combined environments.

Trait†	Environment	Mean‡ ± S.E.	L.S.D. (0.05)	C.V. (%)	σ^2_G ± S.E.	σ^2_P	σ^2_E	σ^2_{GE}	σ^2_ε	H	H C.I.
DMA	CSI99	69.64[a] ± 1.45	4.04	2.90	11.04 ± 1.41	13.13	.	.	4.07	0.84	(0.80,0.88)
DMA	BEI99	71.50[a] ± 1.86	5.20	3.61	9.65 ± 1.43	13.12	.	.	6.65	0.74	(0.66,0.79)
DMA	CSI00	57.68[b] ± 1.10	3.08	2.60	4.13 ± 0.61	5.35	.	.	2.26	0.77	(0.71,0.82)
DMA	Combined	66.43 ± 1.19	1.58	3.15	6.27 ± 0.13	7.67	54.05	1.76	4.38	0.82	(0.77,0.85)
DMA	CSH00	83.04[a] ± 1.40	3.90	2.35	2.95 ± 0.52	4.90	.	.	3.82	0.60	(0.49,0.69)
DMA	TAH00	68.44[c] ± 0.93	2.59	1.92	1.87 ± 0.29	2.73	.	.	1.72	0.68	(0.60,0.75)
DMA	CSH01	71.82[b] ± 0.95	2.65	1.85	1.96 ± 0.32	2.86	.	.	1.77	0.68	(0.59,0.76)
DMA	Combined	74.46 ± 0.71	0.56	2.10	2.06 ± 0.04	2.56	59.89	0.23	2.44	0.80	(0.76,0.84)
PAN	CSI99	1.33E5[a] ± 2.34E4	6.53E4	24.59	1.49E9 ± 2.14E8	2.00E9	.	.	1.06E9	0.73	(0.65,0.79)
PAN	BEI99	6.67E4[b] ± 1.63E4	4.54E7	33.84	4.77E8 ± 8.04E7	7.41E8	.	.	5.09E8	0.64	(0.54,0.72)
PAN	CSI00	8.13E4[b] ± 1.39E4	3.89E4	23.43	4.80E8 ± 7.53E7	6.74E8	.	.	3.63E8	0.71	(0.63,0.78)
PAN	Combined	9.39E4 ± 1.54E4	2.20E4	27.25	4.36E8 ± 9.90E6	6.71E8	1.22E9	3.46E8	6.55E8	0.65	(0.57,0.72)
PAN	CSH00	1.03E5[b] ± 1.50E4	4.18E4	20.48	1.97E8 ± 4.46E7	4.21E8	.	.	4.42E8	0.47	(0.32,0.58)
PAN	CSH01	1.76E5[a] ± 2.27E4	6.33E4	18.14	4.43E8 ± 1.05E8	9.56E8	.	.	1.02E9	0.46	(0.31,0.58)
PAN	Combined	1.37E5 ± 1.71E4	1.89E4	20.07	1.22E8 ± 6.49E6	4.14E8	2.44E9	1.74E8	7.61E8	0.29	(0.10,0.45)

† Mean values for DMA and PAN in days and units, respectively.
‡ Environment means followed by the same letter are not significantly different from each other at the 0.05 level (L.S.D.).

Tillering Ability. Due to problems during the TAH00 establishment, several rows were not planted. This resulted in several experimental units being reduced in size by one-half. Pre-examination of the data at this specific environment and further across data combination and analysis revealed that parameter estimation was inconsistent as compared to when only the other two environments were included in the analysis. Therefore, PAN data from TAH00 was eliminated from overall analysis.

With the exception of the combined TCH, there were highly significant ($P < 0.0001$) differences among genotypes in the RIL and TCH at individual and across environments (Table 11). RIL in CSI99 produced almost 100% more panicles per unit area than in BEI99. Although differences in PAN between BEI99 and CSI00 were as large as 15,000 panicles per ha, this difference was not statistically important (Table 10). Ability to tiller is greatly correlated to environmental conditions during the early phases of the growing season. Harsh conditions at planting reduce stand establishment and thinner stands promote additional tillering. Conversely, higher plant densities inhibit tillering. In addition, the environmental conditions affect tillering with cooler, moist weather promoting additional tillering.

Heritability estimates for PAN in the RIL and TCH from this study are variable compared to those reported by Sanchez-Gomez (2002), and higher than those reported by Liang et al. (1969) and Chung and Liang (1970). While heritability for PAN in RIL was high (0.65) when individual environmental data was combined, TCH *H* was quite low (0.29). However, individual analyses of TCH showed that PAN heritabilities in CSH00 and CSH01 were 0.47 and 0.46. This implies that experimental design failed to

Table 11. Analysis of variance for PAN at individual and combined environments for RIL and TCH.

Env	Source	df	M.S.	Variance Component Coefficients $\sigma^2_R / \sigma^2_{R(E)}$	σ^2_G	σ^2_E	σ^2_{GE}
CSI99	R	1	4.61E9*	174.00	.	.	.
	G	184	3.89E9**	.	1.94	.	.
	Error	173	1.06E9				
BEI99	R	1	1.13E10**	169.00	.	.	.
	G	182	1.43E9**	.	1.92	.	.
	Error	168	5.09E8				
CSI00	R	1	1.49E9	159.00	.	.	.
	G	181	1.26E9**	.	1.87	.	.
	Error	158	3.63E8				
Combined RIL	R(E)	3	5.49E9**	167.33	.	.	.
	G	185	3.72E9**	.	5.54	.	1.87
	E	2	4.13E11*	167.19	.	334.37	1.84
	GxE	362	1.31E9**	.	.	.	1.90
	Error	499	6.55E8				
CSH00	R	1	5.4E10**	177.00	.	.	.
	G	181	8.3E8**	.	1.97	.	.
	Error	176	4.42E8				
CSH01	R	1	2.27E7	164.00	.	.	.
	G	165	1.90E9**	.	1.99	.	.
	Error	163	1.02E9				
Combined TCH	R(E)	2	2.79E10**	174.50	.	.	.
	G	185	1.57E9*	.	3.79	.	1.98
	E	1	8.43E11*	167.04	.	334.08	1.97
	GxE	169	1.11E9*	.	.	.	1.98
	Error	347	7.61E8				

** Significantly different from zero at the 0.0001 probability level.
* Significantly different from zero at the 0.05 probability level.

properly control exogenous sources of variation; these negatively affected the proper estimation of the genetic parameter when environmental data was combined (Table 10). Therefore, it appears that PAN data should not be combined to output overall adjusted means for further utilization.

Panicle Length and Exsertion. Due to problems at harvest, no panicle length data was collected for TCH in Taft, Texas. Thus TCH combined data for this trait only includes data from CSH00 and CSH01.

RIL and TCH greatly differed for PLE and PEX at all environments and combined analyses, with the exception of CSH01, where no differences were detected in panicle length among testcrosses at the 0.05 level (Tables 12 and 13). No differences among environments were detected in PLE and PEX in both populations. This was unexpected since the environments where the RIL were evaluated generally have a great effect on these two panicle traits. RIL genotype by environment interaction was significant ($P < 0.05$) for both traits, while it was only significant for PEX in TCH.

Genetic correlations between any two RIL environments ranged between 0.69 and 0.99 for PEX, and between 0.35 and 0.95 for PLE. These positive correlations imply that a large panicle with adequate exsertion in any single environment, will likely produce similar results in the other two environments. However, the results were different in the TCH, as genetic correlation for PEX between CSH00 and TAH00 was only 0.12.

Table 12. Analysis of variance for PLE at individual and combined environments for RIL and TCH.

Env	Source	df	M.S.	Variance Component Coefficients $\sigma^2_R / \sigma^2_{R(E)}$	σ^2_G	σ^2_E	σ^2_{GE}
CSI99	R	1	0.30	173.00	.	.	.
	G	184	15.09**	.	1.94	.	.
	Error	172	3.49**				
BEI99	R	1	180.66**	168.00	.	.	.
	G	182	14.03**	.	1.92	.	.
	Error	167	6.01				
CSI00	R	1	0.51	159.00	.	.	.
	G	181	17.04**	.	1.87	.	.
	Error	158	7.98				
Combined RIL	R(E)	3	179.04**	167.00	.	.	.
	G	185	84.71**	.	5.53	.	1.87
	E	2	892.87	166.90	.	333.80	1.83
	GxE	362	17.71**	.	.	.	1.89
	Error	498	12.75				
CSH00	R	1	15.33	177.00	.	.	.
	G	181	11.52*	.	1.97	.	.
	Error	176	7.63				
CSH01	R	1	38.08	164.00	.	.	.
	G	166	7.86	.	1.98	.	.
	Error	163	6.26				
Combined TCH	R(E)	2	26.08*	174.50	.	.	.
	G	185	12.07**	.	3.79	.	1.98
	E	1	16.84	167.06	.	334.13	1.95
	GxE	170	7.13	.	.	.	1.97
	Error	347	6.87				

** Significantly different from zero at the 0.0001 probability level.
* Significantly different from zero at the 0.05 probability level.

Table 13. Analysis of variance component coefficients for PEX at individual and combined environments for RIL and TCH.

Env	Source	df	M.S.	Variance Component Coefficients $\sigma_R^2/\sigma_{R(E)}^2$	σ_G^2	σ_E^2	σ_{GE}^2
CSI99	R	1	447.98**	173.00	.	.	.
	G	184	48.86**	.	1.94	.	.
	Error	172	15.04				
BEI99	R	1	59.86*	169.00	.	.	.
	G	182	47.94**	.	1.92	.	.
	Error	168	12.09				
CSI00	R	1	29.30	159.00	.	.	.
	G	181	28.73**	.	1.87	.	.
	Error	158	10.96				
Combined	R(E)	3	179.04**	167.00	.	.	.
RIL	G	185	84.71**	.	5.53	.	1.87
	E	2	892.87	166.90	.	333.80	1.83
	GxE	362	17.71**	.	.	.	1.89
	Error	498	12.75				
CSH00	R	1	1241.50**	177.00	.	.	.
	G	181	30.62*	.	1.97	.	.
	Error	176	21.19				
TAH00	R	1	7.72	182.00	.	.	.
	G	181	15.08**	.	2.00	.	.
	Error	181	9.44				
CSH01	R	1	466.48**	164.00	.	.	.
	G	166	32.25**	.	1.98	.	.
	Error	163	17.40				
Combined	R(E)	3	571.90**	174.33	.	.	.
TCH	G	181	37.81**	.	5.78	.	1.98
	E	2	3643.04	171.87	.	343.73	1.97
	GxE	347	19.59*	.	.	.	1.98
	Error	520	15.91				

** Significantly different from zero at the 0.0001 probability level.
* Significantly different from zero at the 0.05 probability level.

Significant differences were detected between parental lines and parental testcrosses in the combined analyses of PLE and PEX ($P < 0.05$). RTx7000 averaged a panicle exsertion of 7.62 cm, while RTx430 averaged 5.08 cm. The same trend was observed in their testcrosses, since ATx2752 x RTx7000 had a panicle exsertion that was 3.21 cm longer than the RTx430 testcross. RTx7000 and its testcross had longer panicles than RTx430; RTx7000 averaged panicles that were almost 10% longer than RTx430 panicles, while differences were more subtle in the parental testcrosses, averaging 2.5%.

Although several transgressive segregants were detected for PEX in the RIL, it is not an objective of a sorghum breeding program to select genotypes with extreme exsertions. Panicle exsertions of any desirable line or hybrid should be in the proximity of 15 cm. Thus, RIL genotypes with 15 cm exsertions should be aimed for selection. As mentioned before, neither parental line has a desirable panicle exsertion. However, seven RIL were identified as having exsertions that did not significantly differ from the desirable 15 cm length. In TCH the same trend was observed. Parental testcrosses had better exsertions but were statistically lower than 15 cm. Nevertheless, 10% of the TCH were identified as having an outstanding panicle exsertion (13.63 cm – 16.43 cm). There was some consistency between PEX in RIL and TCH. If RIL with superior exsertion are selected and crossed to ATx2752, about 50% of the resulting testcrosses will have good exsertion. However, 87% of the RIL were classified as having poor exsertion but their testcrosses averaged 15 cm in exsertion. Therefore, in hybrid breeding programs, panicle exsertion should be measured or considered in line per se evaluations, but hybrid evaluation must be used to determine panicle exsertion.

Table 14. PEX and PLE estimated parameters for the RIL and TCH populations by individual and combined environments.

Trait†	Environment	Mean‡ ± S.E.	L.S.D. (0.05)	C.V. (%)	σ^2_G ± S.E.	σ^2_P	σ^2_E	σ^2_{GE}	σ^2_ε	H	H C.I.
PEX	CSI99	6.61[a] ± 2.79	7.78	58.72	17.48 ± 2.71	25.25	.	.	15.04	0.69	(0.61,0.76)
PEX	BEI99	7.48[a] ± 2.51	7.00	46.46	18.64 ± 2.70	24.93	.	.	12.09	0.75	(0.68,0.80)
PEX	CSI00	4.21[a] ± 2.42	6.76	78.67	9.49 ± 1.71	15.34	.	.	10.96	0.62	(0.51,0.70)
PEX	Combined	6.12 ± 1.79	1.91	58.33	12.11 ± 0.24	15.30	2.12	2.62	12.75	0.79	(0.74,0.83)
PEX	CSH00	6.36[a] ± 3.28	9.15	72.37	4.78 ± 1.65	15.52	.	.	21.19	0.31	(0.11,0.46)
PEX	TAH00	10.97[a] ± 2.17	6.06	28.00	2.82 ± 0.79	7.54	.	.	9.44	0.37	(0.20,0.51)
PEX	CSH01	12.81[a] ± 2.96	8.28	32.57	7.49 ± 1.79	16.27	.	.	17.40	0.46	(0.30,0.58)
PEX	Combined	9.98 ± 1.84	1.58	39.98	3.15 ± 0.06	6.54	8.92	1.86	15.91	0.48	(0.36,0.58)
PLE	CSI99	31.42[a] ± 1.34	3.75	5.94	6.00 ± 0.84	7.80	.	.	3.49	0.77	(0.70,0.82)
PLE	BEI99	26.30[b] ± 1.77	4.94	9.32	4.18 ± 0.80	7.32	.	.	6.01	0.57	(0.45,0.67)
PLE	CSI00	26.00[b] ± 2.06	5.77	10.86	4.84 ± 1.02	9.10	.	.	7.98	0.53	(0.40,0.64)
PLE	Combined	27.95 ± 1.14	1.03	8.59	4.31 ± 0.09	5.61	9.34	0.75	5.76	0.77	(0.71,0.81)
PLE	CSH00	26.80[a] ± 1.97	5.49	10.31	1.97 ± 0.62	5.84	.	.	7.63	0.34	(0.15,0.48)
PLE	CSH01	26.57[a] ± 1.78	4.96	9.41	0.81 ± 0.44	3.96	.	.	6.26	0.20	(0.00,0.38)
PLE	Combined	26.70 ± 1.37	0.52	9.82	1.30 ± 0.04	3.18	0.00	0.13	6.87	0.41	(0.24,0.54)

† Mean values for PEX and PLE in cm.

‡ Environment means followed by the same letter are not significantly different from each other at the 0.05 level (L.S.D.).

Sixteen RIL and TCH had panicles that were statistically longer ($P < 0.05$) than that of RTx7000 and its testcross. When individual environmental data for PLE was examined, only one TCH had a panicle length that was statistically longer ($P < 0.05$) than that of RTx7000. The same trend observed for PEX was observed for PLE; 56% of the RIL with the longest panicles resulted in long panicle hybrids. However, only 18% of the long panicle hybrids were derived from lines per se with long panicles. PEX has no causal effect on grain produced by the panicle, just in the quality and weight of the grain harvested from each panicle. However, PLE has a positive genetic correlation with GYL in hybrid combination. Thus, it is imperative that care is exercise when selecting against lines with regular panicle length, since it is quite possible that in hybrid combination such lines result in hybrid with long panicles, consequently higher yields.

No other reports on the heritability of panicle length are reported in the literature. In the current study, heritabilities estimated for PLE and PEX in RIL were 0.77 and 0.79, respectively (Table 14). The same estimates in TCH were 53% and 60% lower than those of the RIL. These estimates increased in both populations when data was combined across environments. Several authors have suggested that more than one environment is necessary to adequately estimate unbiased genetic variances (Comstock and Robinson, 1952; Johnson et al., 1955). By combining PLE and PEX data across environments, better estimation of variance parameters were achieved, resulting in unbiased heritability estimates for both traits.

Panicle Weight. Although differences were detected among genotypes and environments in each of the populations evaluated (Table 15), the genotype by environment interaction was only significant ($P < 0.0001$) in the TCH population. Therefore, no major shifts in ranks were observed in the RIL across environments.

RIL panicles were heavier in CSI00, averaging 68% and 89% more weight than the panicles in CSI99 and BEI99 (Table 16). Overall means in the TCH were 54, 29 and 25 grams per panicle in CSH00, TAH00 and CSH01. As expected, overall TCH panicle weights were higher than RIL. However, PWE observed in CSH00 was significantly higher than those in TAH00 and CSH01. This inconsistency is not the result of higher yields observed in CSI00 (Table 7) compared to TCH performance, but that of thinner plots (Table 10) in the RIL environment.

In lines per se, RTx7000 panicles were heavier than those of RTx430; however, when crossed to ATx2752 the panicles of the RTx430 testcross were significantly heavier than those of RTx7000 testcrosses ($P < 0.05$). Several RIL had greater PWE than RTx7000, but none of these lines produced hybrids that had heavier panicles than those of ATx2752 x RTx430. These results are in agreement with the GYL results that showed that none of TCH yielded more than the RTx430 parental testcross. However, as mentioned before, several TCH produced more tillers per unit area than the RTx430 testcross. It is quite evident that RTx430 excellent combining ability does not rely on transmission of enhanced tillering ability of the main plants for enhanced performance, but on specific traits that increase productivity of the main panicle.

Table 15. Analysis of variance for PWE at individual and combined environments for RIL and TCH.

Env	Source	df	M.S.	Variance Component Coefficients $\sigma^2_R / \sigma^2_{R(E)}$	σ^2_G	σ^2_E	σ^2_{GE}
CSI99	R	1	2045.44**	174.00	.	.	.
	G	184	119.67**	.	1.94	.	.
	Error	173	63.89				
BEI99	R	1	3100.48**	169.00	.	.	.
	G	182	86.20**	.	1.92	.	.
	Error	168	40.52				
CSI00	R	1	939.50*	156.00	.	.	.
	G	181	252.52**	.	1.86	.	.
	Error	155	95.48				
Combined	R(E)	3	2028.47**	166.33	.	.	.
RIL	G	185	2.46**	.	5.51	.	1.86
	E	2	19.38*	166.26	.	332.51	1.83
	GxE	362	1.53	.	.	.	1.89
	Error	496	65.85				
CSH00	R	1	304.04*	177.00	.	.	.
	G	181	156.77**	.	1.97	.	.
	Error	176	72.37				
TAH00	R	1	0.62	182.00	.	.	.
	G	181	71.72	.	2.00	.	.
	Error	181	61.10				
CSH01	R	1	36.67	163.00	.	.	.
	G	165	36.67**	.	1.98	.	.
	Error	162	22.20				
Combined	R(E)	3	126.28	174.00	.	.	.
TCH	G	181	1.52**	.	5.76	.	1.98
	E	2	85033.89**	171.45	.	342.89	1.97
	GxE	346	76.79**	.	.	.	1.98
	Error	519	52.78				

** Significantly different from zero at the 0.0001 probability level.
* Significantly different from zero at the 0.05 probability level.

Table 16. PWE estimated parameters for the RIL and TCH populations by individual and combined environments.

Trait†	Environment	Mean‡ ± S.E.	L.S.D. (0.05)	C.V. (%)	σ_G^2 ± S.E.	σ_P^2	σ_E^2	σ_{GE}^2	σ_ε^2	H	H C.I.
PWE	CSI99	26.03[b] ± 5.74	16.02	30.70	28.75 ± 6.59	61.68			63.89	0.47	(0.32,0.58)
PWE	BEI99	23.21[b] ± 4.59	12.82	27.43	23.75 ± 4.86	44.82			40.52	0.53	(0.40,0.63)
PWE	CSI00	43.82[a] ± 7.17	20.04	22.30	84.59 ± 15.35	136.03			95.48	0.62	(0.51,0.71)
PWE	Combined	30.82 ± 4.28	5.11	26.33	26.68 ± 0.59	44.92	114.10	18.61	65.85	0.59	(0.50,0.67)
PWE	CSH00	54.25[a] ± 6.06	16.91	15.68	42.79 ± 8.43	79.48			72.37	0.54	(0.41,0.64)
PWE	TAH00	28.77[b] ± 5.53	15.42	27.17	5.31 ± 3.75	35.86			61.10	0.15	(0.00,0.33)
PWE	CSH01	25.11[c] ± 3.35	9.35	18.76	7.30 ± 2.04	18.50			22.20	0.39	(0.22,0.53)
PWE	Combined	36.32 ± 3.65	4.03	20.00	6.91 ± 0.21	20.23	247.55	12.11	52.78	0.34	(0.19,0.47)

† Mean values for PWE in g panicle^{-1}.

‡ Environment means followed by the same letter are not significantly different from each other at the 0.05 level (L.S.D.).

PWE heritability estimates in RIL are similar to those reported for GYL (Table 16). Estimates reported for the same trait in other populations are larger (Liang et al, 1969; Chung and Liang, 1970). In the current study, PWE was calculated using all the panicles harvested in the plot, including main and tiller panicles. Other authors do not explain how head weight was calculated, and it is possible that only main panicles were utilized for calculations. This could explain differences in heritability estimates.

Trait Correlations

While higher yields were associated with earliness in RIL, fuller season hybrids tended to be more productive (Tables 17 and 18). Also, a strong positive correlation was detected between length of the panicle and grain yield in hybrids, whereas none was detected in the RIL.

While grain yield was positively correlated with panicle weight, panicle number, and plant height in RIL, only the correlation with plant number was greater than 0.60 (Table 17). The same trend was observed in TCH, with correlations higher than 0.52 for the same traits (Table 18). Lothrop et al. (1985b) reported a similar association between GYL and PAN. These results are logical since it is expected that the greater the number of heavier panicles produced by a specific genotype, the higher the grain yield.

Table 17. RIL combined genotypic† (r_G) and phenotypic‡ (r_P) correlation coefficients among seven traits and their standard errors§ across three environments.

Trait	PWE	GYL	PAN	DMA	PHE	PEXS	PLE
PWE		0.39	-0.34	0.08	-0.02	-0.17	0.12
		0.03	*0.03*	*0.04*	*0.04*	*0.04*	*0.04*
GYL	0.26		0.60	-0.21	0.31	0.15	0.06
	0.12		*0.02*	*0.04*	*0.04*	*0.04*	*0.04*
PAN	-0.57	0.65		-0.35	0.34	0.36	-0.06
	0.10	*0.07*		*0.04*	*0.04*	*0.04*	*0.04*
DMA	0.24	-0.22	-0.44		-0.15	-0.37	0.18
	0.10	*0.11*	*0.08*		*0.05*	*0.04*	*0.04*
PHE	-0.05	0.39	0.37	-0.16		0.41	0.22
	0.11	*0.10*	*0.09*	*0.09*		*0.04*	*0.04*
PEX	-0.36	0.18	0.50	-0.49	0.56		-0.15
	0.10	*0.11*	*0.08*	*0.07*	*0.07*		*0.04*
PLE	0.25	0.09	-0.17	0.38	0.28	-0.19	
	0.11	*0.11*	*0.10*	*0.08*	*0.09*	*0.09*	

† Below diagonal.
‡ Above diagonal.
§ Denoted in italics below each correlation coefficient.

Dalton (1967) suggested that sorghum hybrids with taller plants tend to have higher yields. Higher yields are due to a greater number of leaves produced in taller genotypes which increases light interception and consequently increases photosynthate production. Higher photosynthate production and translocation to the grain results is higher yields. If environmental conditions are conducive, fuller season hybrids tend to yield more since light interception is extended for a longer period of time, while on the

contrary, RIL lack the genetic potential to be more productive as the growing period extends.

Table 18. TCH combined genotypic† (r_G) and phenotypic‡ (r_P) correlation coefficients among seven traits and their standard errors§ across three environments.

Trait	PWE	GYL	PAN	DMA	PHE	PEXS	PLE
PWE		0.25	-0.47	0.32	-0.09	-0.56	0.67
		0.03	*0.03*	*0.03*	*0.18*	*0.18*	*0.26*
GYL	0.65		0.59	0.02	0.19	0.05	0.65
	0.20		*0.03*	*0.04*	*0.03*	*0.17*	*0.32*
PAN	-0.51	0.52		-0.29	0.26	0.31	-0.05
	0.26	*0.28*		*0.04*	*0.04*	*0.04*	*0.04*
DMA	0.62	0.31	-0.32		-0.06	-0.21	0.15
	0.13	*0.12*	*0.18*		*0.04*	*0.03*	*0.04*
PHE	0.15	0.53	0.56	0.02		0.40	0.10
	0.18	*0.12*	*0.21*	*0.10*		*0.03*	*0.04*
PEX	-0.56	0.05	0.69	-0.38	0.61		-0.27
	0.18	*0.17*	*0.21*	*0.11*	*0.10*		*0.04*
PLE	0.67	0.65	-0.34	0.34	0.18	-0.27	
	0.26	*0.32*	*0.29*	*0.13*	*0.15*	*0.16*	

† Below diagonal.
‡ Above diagonal.
§ Denoted in italics below each correlation coefficient.

Sanchez-Gomez (2002) reported that panicle weight is the trait with the highest association with grain yield, but the results of the current study indicate that while a positive association exists between the traits, it is not strong enough to be considered of

great importance. Therefore, selecting lines for higher panicle weights will not necessarily result in selecting a genotype with higher yield potential. There are other traits that offer better causal relationships with GYL, such as panicle number and plant height.

Conclusions

No major differences were detected between different statistical procedures that were utilized to estimate genetic, environmental and residual variances in the individual environmental analyses, as well as in the combined analyses of all seven different traits in both populations. Subtle differences were detected between models when means were adjusted for missing observations and/or sources of variations. Generally, adjustment provided by GLM was less drastic than that of MIXED. Since the primary objective of the analyses described in the chapter was to obtain the "best" genotype means for further utilization in genotypic analysis, care was exercised in mean adjustment. Therefore, GLM was chosen for further analysis. However, if the objective had only been to obtain heritability estimates, both models produce similar if not identical estimates.

Enough genetic variability was detected among lines in the recombinant line population. Superior transgressive segregants were identified for each trait in the RIL; yet, when these superior genotypes were testcrossed, the resulting hybrids were not

statistically better performers than the best parental testcross. RTx430 possesses such unique genetic complexion, that none of these derived lines were able to outperform RTx430 in hybrid combination. However, the unique genetic complementation and interaction observed between RTx430 and ATx2752 genes, may not be observed when this restorer line is crossed to another tester.

As expected, reductions in genetic variation were observed in harsher environments. However, the highly positive GYL correlation (r_G = 0.72) observed between stress and non-stress environments suggests that the selection of superior genotypes may be done at either location. Nonetheless, lines evaluated in this research are advanced generation genotypes with a very low number of loci still segregating. Non-additive and epistatic effects still in play at early generations may have a greater effect on the stability of the genotypes across these type of environments.

Heritability estimates reported herein are in agreement with those found in the literature, with very few exceptions. Causal relationships were detected between traits evaluated. Taller plants tend to yield more as lines per se and as hybrids. A positive correlation between the height of RIL and TCH may allow for an indirect selection for higher yield in hybrid combination by selecting taller genotypes during the breeding process. Indirect selection responses need to be estimated between plant height and grain yield to be more conclusive about this matter.

CHAPTER IV

GENETIC MAPPING AND ANALYSIS OF QUANTITATIVE TRAIT LOCI IN SORGHUM FOR GRAIN YIELD AND SIX YIELD RELATED TRAITS

Introduction

Many of the most important plant traits, such as grain yield and its components, are quantitatively inherited. The advent of molecular markers has allowed scientists to dissect the genome of several crop species which has greatly facilitated the study of such traits, with the ultimate objective of improving plant breeding processes. While molecular genetics provides great potential, it has not yet been as effective as traditional plant breeding for quantitatively inherited traits. Plant breeders have started to greatly benefit from the utilization of molecular genetic maps and statistical tools to define important genetic regions and even isolate agronomically important genes. For quantitative traits, the dissection of complex traits into simple genetic factors tagged by molecular markers should suffice (Rami et al., 1998).

In sorghum several linkage maps have been developed throughout the years and some of these have been used to identify molecular markers linked to genetic loci of agronomic importance (Subudhi and Nguyen, 2000). The information generated by these genetic maps will be of vital importance for plant improvement, linking information

derived by plant breeders and plant biology scientists that will allow the identification and insertion of useful agronomic genes into cultivars, positional cloning of genes, possible widening of genetic pool through comparative genomics among related and unrelated species, as well as elucidation of complex biological processes directly related to superior agronomic performance in elite germplasm (Pereira and Lee, 1995).

The first reports of genomic regions associated with grain yield per se, and grain yield components were reported by Tuinstra et al. (1996). Paterson et al. (1998) located nine and four QTL correlated to phenotypic variation of seed size and number, respectively. A major chromosomal region involved in "grain yield components" was identified on LGA in a *Sorghum caudatum* x *guinea* RIL population (Rami et al., 1998). Presence of dwarfing genes on LGA with pleiotropic effects on morphological and productivity traits may be directly responsible for the detection of multiple QTL in this genomic region. Molecular markers linked to major QTL may be utilized to fix these regions in breeding populations in early stages of development. However, molecular breeders should focus on identifying QTL with smaller but specific effects and fixing them in the germplasm, since their effects are more difficult to identify and fix through classical breeding. Unfortunately, trait loci with minor effects are also the most difficult to identify using QTL analysis.

Most QTL studies have been completed on wide cross populations from which very little useful information can be derived in regards to agronomically quantitative traits, such as yield. In most cases, there were no attempts to determine the consistency of detected QTL in line per se and hybrid combination.

The objectives of this research are (1) to identify QTL responsible for the phenotypic variation of grain yield and six yield related traits in a recombinant inbred line (RIL) population, (2) to determine the consistency of the detected QTL in hybrid combination, and (3) to determine if there are any genomic regions with pleiotropic effects on two (or more) different traits.

Materials and Methods

Mapping Population

The population of recombinant inbred lines (RIL) derived from the cross of inbred lines RTx430 and RTx7000 used in Chapter III was used for the current research. Details of the production of these RIL are found in Chapter III.

Genotypic Data

Eight to twelve seeds from 182 recombinant inbred lines were planted in small pots and placed in a greenhouse for germination. Fresh plant tissue was harvested by cutting five seedlings 7 to 10 days after emergence. Tissue was placed in plastic containers and frozen with liquid nitrogen. Samples were stored at -80°C until DNA extraction.

DNA was extracted using the FastDNA® Kit (BIO 101 Inc., La Jolla, California). Approximately 0.25 grams of frozen tissue were placed into tubes containing 800 µl of cell lysis DNA solubilizing solution for vegetation (CLS-VF) and 200 µl of protein precipitation solution (PPS). Samples were homogenized by placing the tubes into a FastPrep® instrument. The instrument was run twice for 20 seconds at a speed setting of five. Samples were spun for five minutes at 12,000 g to pellet the debris. 600 µl of supernatant were transferred to new tubes and 600 µl of binding matrix were added to each tube. Samples were gently shaken for five minutes at room temperature, and pulse spun for five seconds to pellet the binding matrix.

Supernatant was discarded and the pellet resuspended with 500 µl of salt-ethanol wash solution (SEWS-M). Supernatant from each sample was centrifuged twice for one minute at 12,000 g in new spin tubes equipped with spin filters. The spin filter was transferred to new tubes and washed with 100 µl of DNA elution solution (DES). After storing samples at room temperature for five minutes, the tubes were centrifuged for one minute at 12,000 g to transfer the DNA to the catch tube. Extracted DNA was quantified by fluoremetry with a Fluorometer TD-360 (Turner Designs Inc.) and diluted to a final concentration of 100 ng μl^{-1} using 0.5x TE buffer (10 mM Tris-Cl, pH 7.5 1 mM EDTA).

Anchor Molecular Markers

Information generated by Menz et al. (2003) on the parents of this RIL population allowed the identification of SSR and specific AFLP primer combinations that would generated polymorphic markers in the recombinant inbred population evaluated in this study (Tables 19 and 20). Based on this information, AFLP primer combinations were utilized to generate anchor markers that established correspondence with the high density linkage map of sorghum (Menz et al., 2002). In addition to these anchor markers, additional AFLP polymorphic markers were obtained to further saturate the linkage map (See discussion below).

Amplified Fragment Length Polymorphism (AFLP) Marker Amplification

AFLP Marker Pre-Amplification and Adapter Ligation. AFLP markers were amplified following the protocol of Vos et al. (1995) as modified by Klein et al. (2000). 500 ng of genomic DNA (5 µl of DNA) were restricted using 0.3 µl *Eco*RI (Pharmacia-stock 17 U μl^{-1}, final concentration 5 U) and 1.25 µl *Mse*I (NEB-stock 4 U μl^{-1}, final concentration 5 U) restriction enzymes in 4 µl of 10X restriction buffer and 29.35 µl of distilled water. DNA was restricted by incubating for two hours in a 37°C water bath. A ligation master mix of 1 µl 5 pmol *Eco*RI adapter, 1 µl 50 pmol *Mse*I adapter, 1 µl 10 mM ATP, 1 µl 1 U T4 DNA ligase, 1 µl 1X RE buffer, and 5 µl distilled water, was added to each restricted DNA sample and incubated at 37°C overnight. Concentrated DNA was diluted to a final concentration of 1 ng μl^{-1} by adding 450 µl 1X TE buffer.

DNA was pre-amplified by adding 2 µl 10X PCR buffer (Promega Corporation, Madison, WI), 2 µl 25 mM $MgCl_2$, 1.6 µl 2.5 mM dNTPs (Pharmacia), 1 µl 30 ng μl^{-1} E+0 preamp primer (GTAGACTGCGTACCAATTC), 1 µl 30 ng μl^{-1} M+C preamp primer (GATGAGTCCTGAGTAA-C), 0.08 µl *Taq* polymerase (Promega Corporation, Madison, WI), and 7.32 µl of distilled water to 5 µl of diluted DNA. Polymerase chain reaction (PCR) was performed in a PE9700 (Perkin-Elmer) DNA Thermal Cycler for 20 cycles of 30 seconds at 94°C, 60 seconds at 56°C, and 60 seconds at 72°C. Final holding temperature was 4°C. Following preamplification, DNA was diluted to a final concentration of 25 pg μl^{-1} by adding 180 µl of 0.5 TE buffer to each reaction. Preamplifications were used as template for selective amplification.

Selective AFLP Amplification. Selective amplification was done by mixing 2 µl (50 pg) of preamplified DNA, 1 µl 10X PCR buffer, 1 µl 25 mM $MgCl_2$, 0.8 µl 2.5 mM dNTPs, 2 µl 7.5 ng μl^{-1} M-selective primer, 0.3 1 µM (1 pmol μl^{-1}) IRD-labeled (LI-COR Inc.) E-selective primer, 0.04 *Taq* polymerase, and 2.86 µl distilled water. PCR was performed by doing a touchdown cycle of 94°C for 2 minutes, followed by 13 cycles of 94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 60 seconds, lowering the annealing temperature by 0.7°C for after each cycle. This was followed by 23 cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 60 seconds, followed by a five minute hold at 72°C, with a final holding temperature of 4°C. Samples were later stored at -20°C for further utilization.

Table 19. *Eco*RI and *Mse*I primer combinations utilized to generate AFLP polymorphic markers.

General Nomenclature	Standard Nomenclature	Anchor Markers	Total Markers
E-ACC + M-CAC	E36M48	9	16
E-ACC + M-CAA	E36M47	4	12
E-ACC + M-CAG	E36M49	5	16
E-ACC + M-CCA	E36M51	1	15
E-ACC + M-CGG	E36M57	4	7
E-ACC + M-CTC	E36M60	6	17
E-ACC + M-CTG	E36M61	2	20
E-ACC + M-CTT	E36M62	7	13
E-AGT + M-CAA	E42M47	2	7
E-AGT + M-CGA	E42M55	6	9
E-CAA + M-CAC	E47M48	7	24
E-CAA + M-CAG	E47M49	4	11
E-CAA + M-CTA	E47M59	5	25
E-CTG + M-CAC	E61M48	3	13
E-GAA + M-CAA	E63M47	7	15
E-GGA + M-CCA	E71M51	3	11
E-GGA + M-CCG	E71M53	3	12
E-GGA + M-CGT	E71M58	5	13
E-GGA + M-CTA	E71M59	4	12
E-GGA + M-CTC	E71M60	2	12
E-GGA + M-CTT	E71M62	1	10
E-TAC + M-CTA	E80M59	3	7
E-TGA + M-CAA	E87M47	6	21
E-TGA + M-CCT	E87M54	3	14
E-TGA + M-CTC	E87M60	3	19
TOTAL		105	351

Table 20. Primer sequence of SSR utilized as anchor markers in the RIL linkage map.

Locus	Type of SSR(s)†	Sequence of Primers 5'-Forward Primer-3' 5'-Reverse Primer-3'
Xtxp8	$(TG)_{31}$	ATA TGG AAG GAA GAA GCC GG AAC ACA ACA TGC ACG CAT G
Xtxp18	$(AG)_{21}$	ACT GTC TAG AAC AAG CTG CG TTG CTC TAG CTA GGC ATT TC
Xtxp96	$(GA)_{24}$	GCT GAT GTC ATG TTC CCT CAC CAT TCG TGG ACT CTG TCG G
Xtxp120	$(AT)_{18}$	AAA GCT CGG CGT TAG AAA TA CGC TTA ACA ACT CCT ACC ATC
Xtxp354	$(GA)_{21}+(AAG)_3$	TGG GCA GGG TAT CTA ACT GA GCC TTT TTC TGA GCC TTG A

† A '+' represents repetitive sequences in SSR that are separated by more than five bases apart.

Simple Sequence Repeat (SSR) Marker Amplification

Based on fluoremetry readings, DNA was diluted to a final concentration of 2.5 ng μl^{-1} using 0.5X TE buffer. SSR marker amplification was done by mixing 1 µl 10X PCR buffer, 0.8 µl 25 mM $MgCl_2$, 0.8 µl 2.5 mM dNTPs, 0.08 µl *Taq* polymerase, 3.32 µl distilled water, 1 µl 1 pmol μl^{-1} forward IRD-labeled primer, 1 µl 1 pmol μl^{-1} reverse primer, with 2 µl of dilute DNA template. PCR was performed by doing an initial DNA denaturation of 94°C for 2 minutes, followed by 26 cycles of 94°C for 60 seconds, 55°C

for 30 seconds, and 72°C for 60 seconds, followed by a ten minute hold at 72°C. Reactions were stored at -20°C.

Amplification Product Analysis

Amplification products were analyzed using a LI-COR model 4200L-2 dual-dye automated DNA sequencing system. This system allowed for the simultaneous analyses of amplification products that were labeled with different dyes, IRD-700 nm and IRD-800 nm.

For gel analysis, amplified products labeled with different IRD were pooled together by mixing equal volumes of reaction (5 µl). To prepare the samples for gel loading, 2 µl of fusion dye (LI-COR) were added to each pooled sample and denatured for 5 minutes at 95°C. After denaturation, 1 µl of each sample was loaded on a 6.5% polyacrylamide 7 M urea gel. Electrophoresis conditions and data collection were as described by Klein et al. (2000).

Linkage Map Construction

AFLP and SSR marker data obtained from the sequencers was analyzed with Bionumerics software (Applied Maths, Kortrijk, Belgium) as described by Klein et al. (2000). Linkage analysis was conducted using Mapmaker/EXP 3.0 (Whitehead Institute, Cambridge, MA). The population was analyzed as a recombinant inbred selfing population (ri-self option). Analyses were commenced by creating 10 linkage groups

(LG) using the “make chromosome” command and assigning 105 AFLP (Table 19) and 5 SSR (Table 20) markers as anchor markers to each LG using the “anchor” command. By means of two point linkage analysis, remaining AFLP loci were sorted into each of the LG using the “assign” command with a LOD 6.0 and a Kosambi distance threshold of 40 centimorgans (cM). Unassigned loci were further analyzed and reassigned by increasing the LOD value.

Loci order within each LG was determined by means of multipoint linkage analysis. With the “error detection” option on, the “compare”, “order”, and “ripple” commands were repeatedly invoked to set up an initial framework map for each LG using the most informative markers. Remaining markers within each LG were then added in decreasing order, based on the value of their information, using the “build” command, and validating the new sequence with the “order” command.

Possible errors were identified with the “genotype” command. Gel images were visually examined and genotyping errors corrected. Data was reanalyzed in Mapmaker/EXP and correct map distances were estimated with the “error detection” option off. Loci sequences within each LG were reexamined with the “order” command before final marker orders were set using the “framework” command for each LG. Unmapped markers were placed into each LG with the “place” command with no effect on map length.

QTL Analyses

QTL analysis was completed for grain yield, plant height, days to mid-anthesis, tillering ability, panicle length, exsertion and weight. The reader is referred to Chapter III for details on field evaluation and data collection. Genotypes with missing observations in at least one of the environments were not utilized in the QTL analyses. This was done to assure that only genotypes evaluated in all environments were utilized to identify QTL.

Composite interval mapping (CIM) was conducted with QTL Cartographer version 2.0 (Statistical Genetics, North Carolina State University, USA). The standard model (Model 6:ZmapQTL) was utilized for QTL detection. Cofactors were chosen using a forward and backward (FB stepwise) regression method with a maximum of five control markers and a window size of 10 cM. A 0.1 p-value was utilized to add and delete markers from the regression model. A 2 cM walking speed was chosen as the precision parameter.

To declare the presence of a QTL, 300 permutations were performed for each trait to determine an α=0.05 genome-wise significance level (Churchill and Doerge, 1994; Doerge and Churchill, 1996). For simplicity of discussion, significant QTL were designated as the locus where the peak was detected. For example, if locus 231 is identified as the peak locus of a grain yield QTL detected in chromosome B, then such QTL was designated QTL 231 in LG B.

Digenic epistatic interactions for all traits were tested between all pairs of loci with two-locus analyses of variance using a SAS® EPISTACY program (Holland, 1998). A significance level of α=0.001 was utilized to test all pairwise interactions. This significance level was calculated by dividing the comparison-wise error rate of 0.05 by *g(g-1)*0.5*, where *g* is the number of chromosomes in the Sorghum genome.

Linear Additive Model Selection

Linear additive model building technique utilized for this study was a modified version of that published by Holland et al. (1997). The percentage of total phenotypic variance (PVE) was determined by fitting a model including all putative QTL and significant epistatic loci for a trait. A linear additive model with putative QTL loci excluding epistasis was obtained for all individual environments experiments, and a full model including QTL loci and epistasis factors was obtained across environments for all traits.

Models including only putative QTL were constructed by fitting all putative QTL within an experiment into a linear regression model using PROC REG included in SAS® 8.0. If two QTL for one trait were detected in the same chromosome, a preliminary analysis was run by fitting the two QTL into the linear model. If both loci remained significant ($P < 0.05$), then both were included in the all QTL model building step. If one of them was not significantly different than zero, then the QTL that explained the greatest variability was selected and advanced to the next step. Best all QTL model was

selected by means of greatest R^2 under the restriction that all factors in the model remained significant ($P < 0.05$) using the options "selection" and "best" included in the same statistical procedure.

Models including epistasis were developed by first constructing all possible models containing an epistasis interaction term and their main effects. The best model was selected as the one with the greatest R^2 and with the interaction remaining significant at the 0.05 level. Then, the main effects and interaction term from the best model were added to the rest of the models being tested, resulting in models with two interaction terms and four main effects. Again, the best model was that with the greatest R^2 and interactions remaining significant. This process was repeated interactively, adding previously selected main effects and interaction terms to the remaining models, until no further model could be developed due to loss of significance of the interaction terms.

Once the best epistasis model was identified, the factors of the best epistasis model were combined with the model containing all putative QTL one at a time. The model with the putative QTL loci and the epistasis factors with the greatest R^2 with QTL loci and interactions significant was chosen as the best model. All remaining epistasis main factors and interactions were added to the best model one at a time, choosing the best model as previously described. This process was repeated until no further epistatic terms could be added to the model while remaining statistically significant. At the end, the best model had putative QTL and epistasis interactions significant ($P < 0.05$) and explained the greatest amount of the total variability observed.

Results and Discussion

Linkage Map

AFLP and SSR primer combinations generated 354 polymorphic markers in the restorer RIL population; however, only 174 marker loci were utilized to construct the linkage map (Table 21). Loci were assigned to 12 different linkage groups. Due to the utilization of anchor markers it was possible to determine that chromosome A and D were each divided in two linkage segments. Unpublished data from Menz et al. (2003) shows that polymorphism exists between the parental lines throughout chromosome A and D which should permits single LG to be obtained for each chromosome. Nevertheless, most of the polymorphic loci located in the gaps in the present map are products of *Pst*I and *Mse*I AFLP primer combinations, which were not utilized in this research.

With the exception of two loci, the order of loci in each LG coincided with that published by Menz et al. (2002). One hundred and eighty loci were either too close to more informative markers or could not be placed to a unique position under the linkage criteria utilized to construct this linkage map and hence, were not incorporated into the framework map.

This linkage map covers 1573 cM with marker loci spaced at an averaged 9.04 cM, with interval distances ranging from 2.1 to 22.8 cM. Menz et al. (2002) utilized a wide cross (BTx623 x IS3620C) to develop their linkage map of 1667 cM. Even though

the parental lines utilized to develop this RIL population are not related by pedigree, both lines should have linkage blocks common in elite restorer lines, thus making this a less diverse cross. However, this map is only 10% shorter than that published by Menz et al. (2002), which suggests an adequate coverage of the Sorghum genome for subsequent QTL analyses.

Fifty eight of the loci utilized to develop the map exhibited significant deviation ($P < 0.05$) from the genotypic ratios expected of 1:1 for a RIL population at this generation level. Sixty four percent of these loci showed segregation distortion towards the RTx7000 alleles. Three main cluster were detected in LG A-b, D-b, and I. Clusters A-b and D-b were in excess of RTx7000 alleles while LG I of RTx430. Detection of strong segregation distortion in one genomic regions suggests selection favoring one parental allele. Klein et al. (2001) reported a QTL for grain mould resistance located in LG D. Since RTx430 is quite susceptible to grain molding, which significantly reduces germination and seedling viability, it may be possible that genotypes with excess of RTx7000 alleles in LG D-b were able to resist better grain mould infections, causing a shift in the normal segregation pattern of the population. The same may be assumed for the other two clusters detected. The excess of alleles from one parent or the other may have allowed the genotypes to endure harsher conditions and to pass their alleles to the next generation causing the detected segregation distortion. Several authors have reported segregation distortions in LGs A and D (Whitkus et al., 1992; Chittenden et al., 1994; Menz et al., 2002). Even though mapping populations utilized by these authors were derived from different parental lines, it is quite clear that genomic regions located

in these linkage groups may possess key genes that have strong effect on general fitness and survival of the individual genotypes.

Table 21. Linkage group designation (LG), recombinant length, and average cM distance between markers of the RIL linkage map.

LG	Length cM	Number of Markers	Average Distance cM
A-a	79.0	8	9.88
A-b	130.1	15	8.68
B	182.8	29	6.30
C	209.6	19	11.03
D-a	21.4	4	5.35
D-b	138.5	12	11.54
E	101.0	11	9.18
F	136.2	12	11.35
G	102.5	13	7.88
H	170.9	18	9.49
I	186.9	19	9.84
J	114.1	14	8.15
TOTAL	1573.0	174	9.04

QTL Analyses

Grain Yield. According to the likelihood ratio test statistic (LR) calculated by the permutation test in CIM, only two QTL located on one chromosome were statistically significant in RIL (Table 22). QTL 214 and 231 located in chromosome B were detected on the QTL analyses of CSI00 and combined, respectively. QTL 214 accounted for 10% PVE, while QTL 231 accounted for 8%. The negative effect at both QTL indicates that alleles from RTx430 were associated with higher grain yields. Since only two QTL could be detected using the permutation LOD, putative QTL were sought by lowering the stringency of the analyses to a LOD of 2.0. Four other QTL were detected on three different chromosomes in all analyses (Figure 2). QTL 239 in LG I, 207 in LG G, 148 in LG C, and 270 in LG I, were detected for CSI99, BEI99, CSI00, and combined, respectively. PVE by individual QTL ranged from 5% to 7%. With the exception of BEI99, QTL detected were associated with higher yields due to RTx7000 alleles in RIL experiments. QTL 214 and 148 explained 15% PVE for CSI00, while QTL 231 and 270 explained 13%.

Epistasis refers to the phenotypic effects of interactions among alleles at multiple loci (Xing et al., 2002). Advent of molecular makers and their utilization has revealed that epistatic interactions play an important role on the genetic basis of quantitative traits (Paterson et al., 1991a; Li et al., 1997). Liao et al. (2001) suggests that there are three types of epistasis affecting complex traits: (1) interactions between QTL, (2) interactions between QTL and modifying loci, and (3) interaction between complementary loci. In this study, 22 digenic epistatic interactions in RIL and 23 in TCH for grain yield were

identified across environments. However, only 11 of these interactions were successfully included in the full additive models and categorized as interactions between complementary loci. Fitness of statistical models was greatly increased by the inclusion of these epistatic terms as explanatory variables. In RIL, just the epistatic interactions accounted for 20% of the total variation observed.

Table 22. Peaks, LOD, R^2, and additive genetic effect of grain yield QTL in the RIL and TCH populations.

Env	LG	Peak Locus†	Position	LOD	R^2‡	Additive
CSI99	I	239	1.72	2.11	0.06	-0.25
BEI99	G	207	0.00	2.37	0.06	0.14
CSI00	**B**	**214**	**1.42**	**3.63**	**0.10**	**-0.42**
	C	148	0.59	2.51	0.07	-0.35
RIL	**B**	**231**	**1.44**	**3.26**	**0.08**	**-0.21**
Combined	I	270	1.76	2.00	0.05	-0.16
CSH00	D-b	99	1.53	2.38	0.08	0.23
TAH00	D-b	135	1.20	2.47	0.06	0.22
	E	348	0.00	2.72	0.07	0.22
	E	**215**	**0.58**	**4.51**	**0.15**	**0.34**
CSH01	B	264	1.17	2.48	0.06	-0.19
TCH	C	344	2.10	2.10	0.05	0.13
Combined	D-b	284	0.04	2.15	0.07	0.14
	G	207	0.08	2.04	0.08	0.15

† Bold lettering indicates significant QTL according to permutation test LR.
‡ Denotes the percentage of phenotypic variation explained (PVE) by the locus.

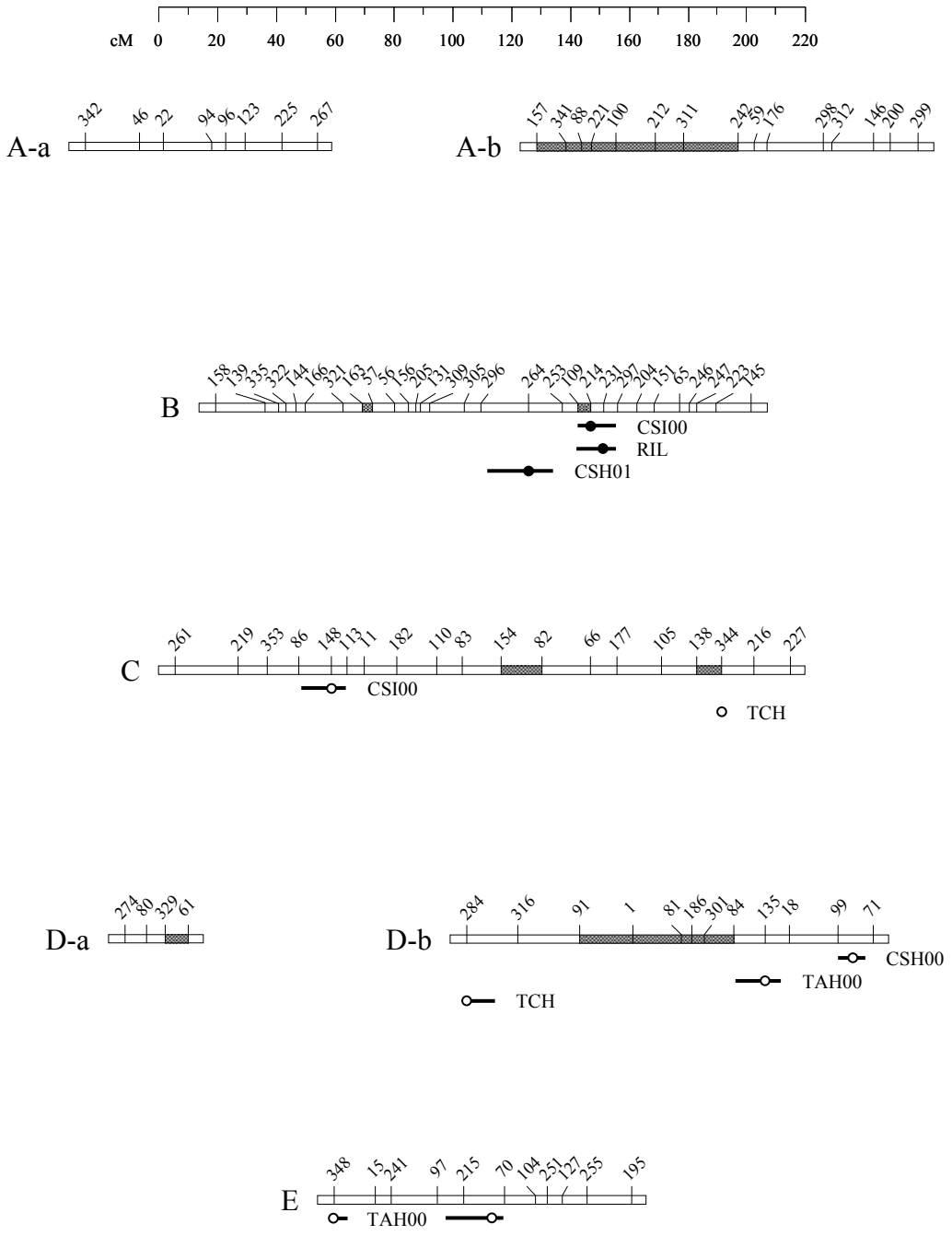

Figure 2. Putative grain yield QTL at individual and across environments. Shaded regions of LG indicate segregation distortion. Bars show positions of QTL with the peak LOD-score identified with a circle. Open circles represent trait QTL affected by the RTx430 allele, while solid circles represent trait QTL affected by the RTx7000 allele.

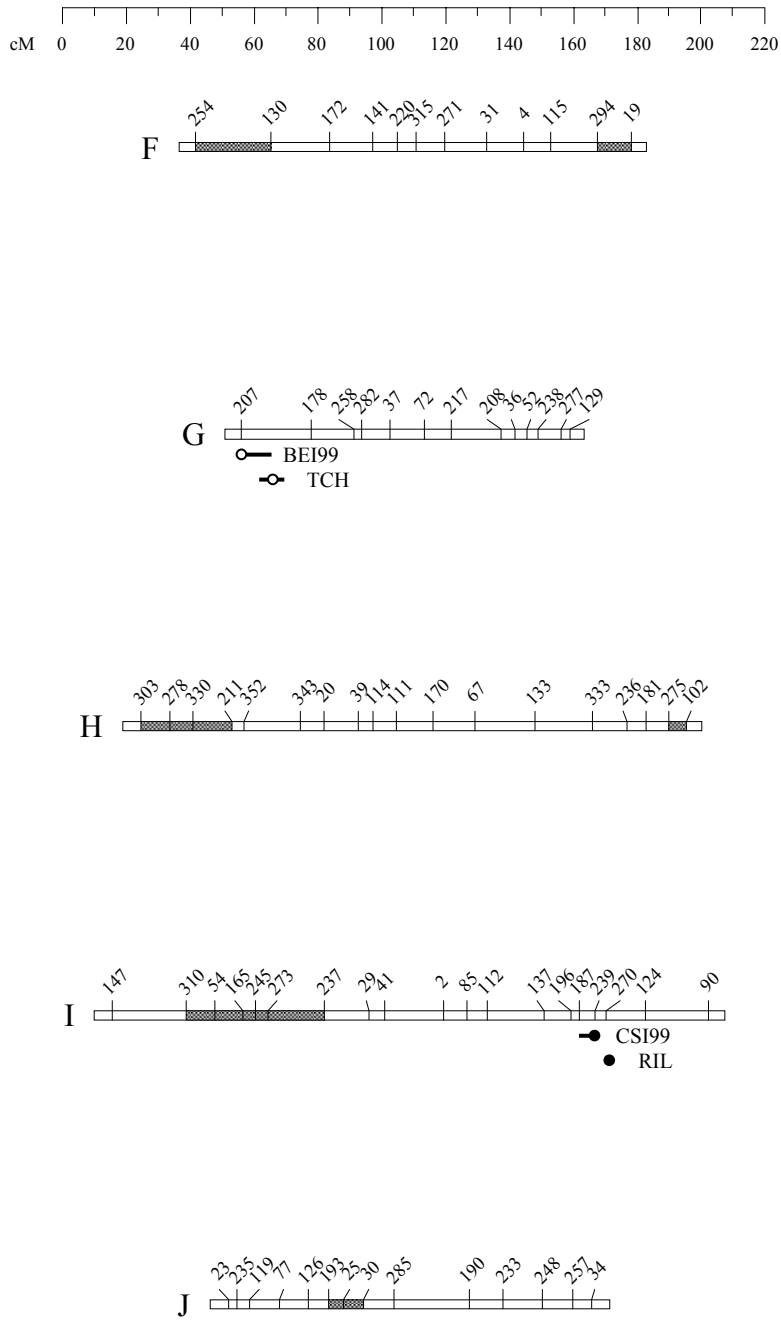

Figure 2. Continued.

In general, genotypes homozygous for RTx430 alleles at both loci yielded less than those genotypes homozygous for alleles from RTx7000. However, better performance was observed in genotypes with combination of homozygous loci than those that had both loci that were homozygous with alleles from the same parental line (Table 23). With the exception of interactions marker loci 11x84 and 225x255, grain yield increased whenever RTx7000 alleles replaced RTx430 alleles in locus A while locus B remained homozygous with RTx430 alleles. The same was observed for genotypes homozygous at both loci for RTx7000 alleles; whenever there was a substitution of RTx7000 alleles at locus A with RTx430 alleles, a slight improvement in agronomic performance was observed.

As in previous studies, QTLs in the RIL population were not consistent across environments. Paterson et al. (1991a) reported that only four of the 29 QTL identified for different traits of tomato were significant across environments. Freyer and Douches (1994) reported that only 20% of the QTL identified were consistent across environments, while Liao et al. (2001) reported that only one panicle number QTL in rice was detected under different growing conditions. Similar results were observed in this study, but there was some consistency between College Station environments and the combined analyses. According to Cardinal et al. (2001), the precision of QTL position becomes very poor when loci exhibit segregation distortion due to incorrect estimates of locus order and biased estimates of recombination frequency when using mapping algorithms of Mapmaker/EXP. Thus, QTL spaced at 20 cM may be considered common for the purpose of comparing them across experiments. QTL 231 detected in

the combined analysis is just 4 cM from QTL 214 detected for CSI00, while QTL 270 in LG I is just 3 cM away from locus 239, which was detected as the QTL peak for CSI99.

Table 23. RIL and TCH digenic epistatic interactions for grain yield across environments.

	Locus			Class Means Loci A,B (MT ha^{-1})			
Env	A	B	R^2	430,430	430,7000	7000,430	7000,7000
RIL	11	84	0.08	2.94	2.55	2.51	2.96
	124	56	0.08	2.26	2.98	2.89	2.80
	129	204	0.08	2.18	2.97	2.85	2.81
	139	156	0.10	2.41	3.07	2.96	2.70
	145	193	0.08	2.29	2.92	3.01	2.83
	225	255	0.09	2.98	2.30	2.77	2.96
TCH	138	145	0.12	5.00	5.19	5.34	4.71
	146	2	0.09	4.99	5.24	5.29	4.86
	223	348	0.09	5.10	5.23	5.32	4.78
	225	344	0.08	5.12	5.21	5.33	4.79

In TCH only one QTL could de detected using the LR output by the permutation test (Table 22). Seven other putative QTL in all analyses could be identified when the LOD was lowered to a threshold of 2. Three different QTL were identified in LG D-b for CSH00, TAH00, and combined analyses (Figure 2). Although they are all on the same linkage group, it is likely that all three are unique because each is more than 20 cM distant from the others. In the combined analyses, individual QTL explained an average

of 6.7% of the phenotypic variation observed, and when all QTL explained 17%. The PVE by all QTL in CSH00, TAH00, and CSH01 was 3%, 9.6%, and 6.9%, respectively. Contrary to what was observed in RIL, combined analysis greatly improved the power of the identified QTL to explain the phenotypic variability observed in grain yield among testcrosses. With the exception of QTL 264 in CSH01, all TCH QTL detected associated higher yields with positive interaction between RTx430 and ATx2752 alleles.

As with the RIL, several significant ($P < 0.001$) epistatic interactions were identified in TCH across environments (Table 23). PVE by epistatic interactions in the combined analysis was 18%. However when epistatic factors were added to the linear models, variation explained by the model increased to almost 50%. It is quite possible that this estimate is inflated although steps necessary to control multicolliniarity were taken. Even though no definitive inferences may be made regarding intra-allelic interactions since genotypic makeup of the tester is unknown, at least it was possible to establish which parental allele combinations improved the performance of the testcrosses. As expected, testcrosses with RTx430 alleles in both loci yielded better than those genotypes with RTx7000 alleles. Nevertheless, better yields were achieved by testcrosses that possessed at least one RTx7000 allele in locus A and at least one RTx430 allele in locus B (Table 23).

Rami et al. (1998) reported a major grain yield QTL in LG A which was not detected in this study. However, grain yield QTL mapping done by Tuinstra et al. (1996) reported one pre-flowering drought and full irrigation yield QTL in LG G an LG C, respectively. RIL evaluated in BEI99 suffered severe drought stress since no

supplemental irrigation was provided and high temperatures were observed throughout the growing season (See chapter III). Interesting enough, a grain yield QTL (QTL 207) was mapped in LG G for this environment in the same genomic region than the QTL reported by Tuinstra et al. (1996) for drought environments. Also in CSI00 where supplemental irrigation was provided as needed, a QTL was detected (QTL 148) that is just a few map units from another small QTL reported by Tuinstra et al. (1996). These results suggest that it is possible that the same agronomic and physiological mechanisms that operate in the drought tolerant parental lines utilized by Tuinstra et al. (1996) to develop the RIL, operate at some extent in RTx430 allowing for the identification of the same QTL under similar environmental conditions. QTL 270 maps to the same genomic region in which Sanchez-Gomez (2002) reported a large (30 cM) grain yield QTL in LG I in a maintainer RIL population. However, the positive effect does not come from the common line in RTx430 and BTx623 pedigrees, since increased yield in the restorer population is due to the presence of RTx7000 alleles in QTL 270 (Table 22), while in the maintainer population, the effect is due to BTx623.

Important genomic regions that contribute to higher grain yield were found in chromosomes D through I, and such regions represent potential targets for molecular breeding. However it is important to mention that several of the loci involved in epistatic interactions were mapped to LG B, meaning that important genes involved in yield expression are located on this chromosome; therefore, this chromosome should be further studied with a higher resolution linkage map and additional robust field studies to determine the extent of its involvement.

While QTL mapping studies have been done in numerous crops and for a wide array of agronomically important traits, most of these studies have been based on the evaluation of lines per se. In hybrid crops such as maize and sorghum, QTL mapping on these type of populations may be of little use for traits that are the result of heterosis, such as grain yield and its components. It is more important to assess which genomic regions are directly related with enhanced performance in hybrid combination, identifying specific genomic regions associated with specific combining ability against one tester, and/or regions associated with general combining ability by testcrossing the inbred population with several testers. As expected, because of the low correlations (r_P = 0.17) between line and hybrid performance reported in the previous chapter, no consistency in QTL detection between RIL and TCH was observed. Different genomic regions are responsible for superior performance of specific RIL genotypes as well as TCH genotypes. By comparing loci involved in epistatic interactions in both populations, two common loci were detected across analyses (Table 23). In these loci positive gene action in inbred line performance of one of the parental line alleles, was detrimental in hybrid combination. Thus, utilization of QTL mapping information derived from inbred lines may be unfavorable for the successful selection of specific and general combiners.

Plant Height. Even though it was highly unlikely that alleles at any of the major dwarfing loci were segregating in the RIL, several QTL (LOD > 2) were detected across experiments (Figure 3). QTL 176 and QTL 312 in LG A-b were detected (LOD > 2.91) in CSI99 and combined analysis of RIL, respectively (Table 24).

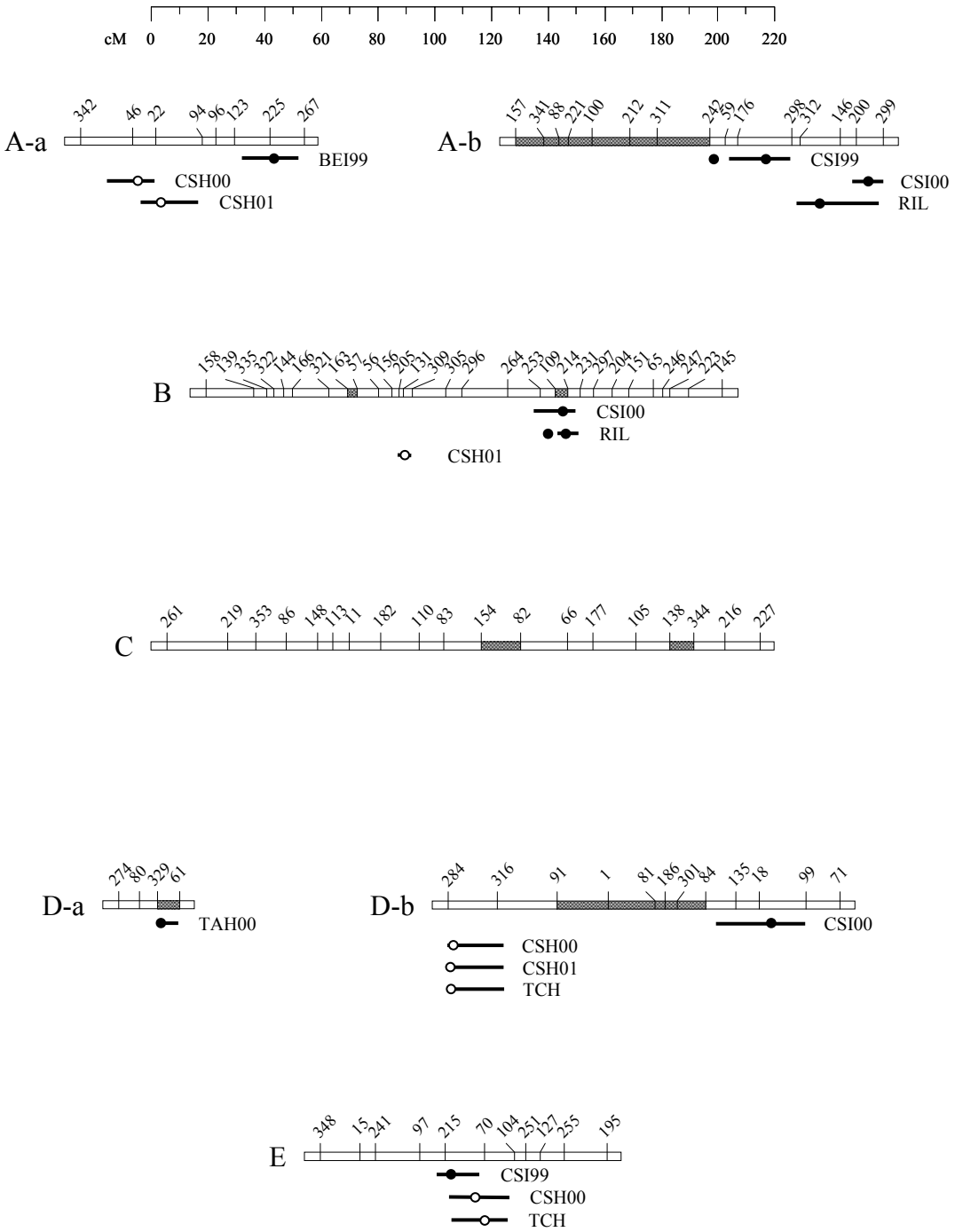

Figure 3. Putative plant height QTL at individual and across environments. Shaded regions of LG indicate segregation distortion. Bars show positions of QTL with the peak LOD-score identified with a circle. Open circles represent trait QTL affected by the RTx430 allele, while solid circles represent trait QTL affected by the RTx7000 allele.

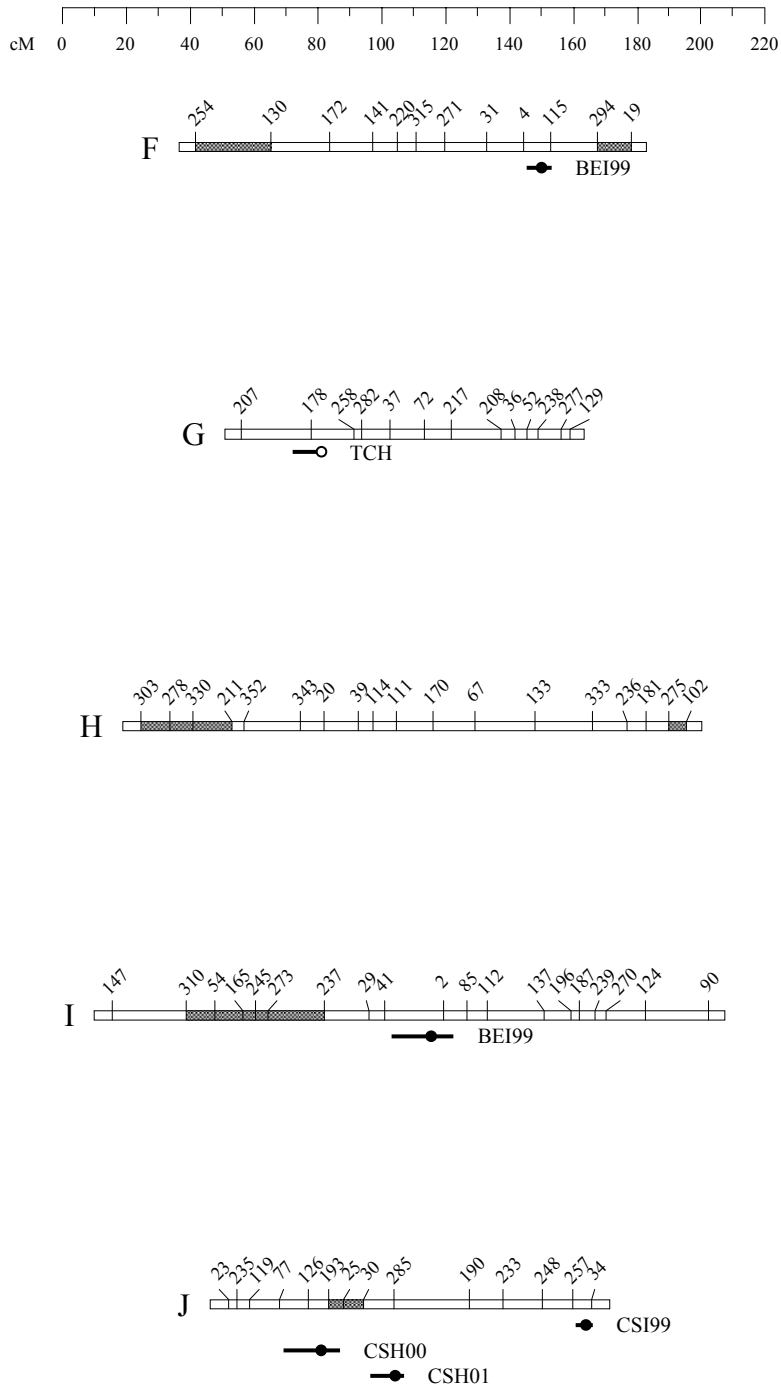

Figure 3. Continued.

Table 24. Peaks, LOD, R^2, and additive genetic effect of plant height QTL in the RIL and TCH populations.

Env	LG	Peak Locus†	Position	LOD	R^2‡	Additive
CSI99	A-b	242	0.79	2.06	0.06	-4.14
	A-b	**176**	**0.95**	**3.32**	**0.14**	**-6.42**
	E	215	0.50	2.21	0.05	3.95
	J	257	1.37	2.21	0.05	3.91
BEI99	A-a	225	0.76	2.50	0.06	-2.89
	F	4	1.23	2.25	0.07	-2.78
	I	41	1.15	2.62	0.09	3.18
CSI00	A-b	200	1.35	2.91	0.07	-4.16
	B	109	1.38	3.12	0.08	-4.69
	D-b	18	1.35	3.08	0.11	-5.33
RIL	**A-b**	**312**	**1.21**	**3.34**	**0.13**	**-4.34**
Combined	B	253	1.32	2.03	0.06	-2.92
	B	109	1.40	2.21	0.06	-3.00
CSH00	A-a	46	0.23	2.69	0.07	2.87
	D-b	**284**	**0.04**	**4.21**	**0.12**	**3.65**
	E	**215**	**0.60**	**4.02**	**0.12**	**3.63**
	J	126	0.33	2.82	0.06	2.75
TAH00	D-a	329	0.15	2.69	0.07	-2.97
CSH01	**A-a**	**22**	**0.31**	**3.50**	**0.08**	**2.54**
	B	131	0.76	2.49	0.05	-2.12
	D-b	**284**	**0.02**	**6.46**	**0.17**	**3.77**
	J	285	0.56	2.12	0.05	1.96
TCH	**D-b**	**284**	**0.00**	**5.72**	**0.14**	**2.74**
Combined	E	70	0.65	2.95	0.07	1.94
	G	178	0.26	2.04	0.05	1.59

† Bold lettering indicates significant QTL according to permutation test LR.
‡ Denotes the percentage of phenotypic variation explained (PVE) by the locus.

Variation in plant height due to QTL 176 CSI99 was strong enough to be detected in the combined analysis for the trait. Such QTL accounted for 13.5% of the variability observed across both analyses, with RTx7000 alleles having a negative effect on the height of the RIL.

Phenotypic variance explained by individual QTL in RIL ranged from 0.05 (QTL 257 in CSI99) to 0.14 (QTL 176 in CSI99). On average, more variation was explained by individual QTL in CSI00, since QTL 200, 109, and 18 explained 7%, 8% and 11%, respectively. When all QTL were considered, 17% PVE was explained in CSI99, 6.4% in BEI99, and 20.3% in CSI00, but in the combined analysis of RIL only 12.3% PVE was explained. Even though two different QTL in LG B were detected in the combined analysis, only QTL 253 could be included in the full model. Closer examination of loci location revealed that locus 253 is only 5.5 cM from locus 109, thus when both loci were included in the full model, they were measuring the same variation resulting in multicolliniarity and PVE overestimation.

Four digenic epistatic interactions were detected for plant height in the RIL (Table 25). Locus 267 was mapped in LG A-a, 264 and 321 in LG B, 91 in LG D-b, 97 in LG E, and loci 111 and 235 in LG H and LG J, respectively. Tallest genotypes were those carrying RTx430 and RTx7000 alleles at loci 91 and 264, respectively. While locus 267 interacted significantly ($P < 0.05$) with loci 97 and 321, the resulting effect of their double interaction was not significant. PVE by each individual interaction varied between 0.07 and 0.11, which is lower than that explained by QTL 312 in LG B. However, when all interactions were added to the PVE full model, R^2 was greatly

increased. Variation explained by epistatic interactions was calculated to be approximately 26%, which is 111% more than that explained by only QTL. As observed for grain yield, epistasis plays an important role in plant height variation among inbred lines evaluated in this study.

In TCH, QTL 284 was consistently detected in College Station environments, and the combined analysis, explaining 12% of the variation observed in CSH00, 17% in CSH01, and 14% across environments (Table 24). According to phenotypic analysis of this trait (see Chapter III), TCH evaluated in College Station were taller than those evaluated in TAH00. Genotypic data analysis supports this observation. Three different QTL in TCH were mapped on genomic regions in chromosome LG A-a, LG D-b, and LG J when they were evaluated at the same location in different years, all with positive effects on plant height coming from RTx430. It is possible that differential expression of genes located within these QTL conferred an advantage to TCH compared to when grown in environments such as TAH00. QTL analysis explained only 7% of the variation for plant height among TCH in Taft, but the PVE for CSH00 and CSH01 was 21.3% and 20.3% respectively. In the combined analysis, QTL analysis explained 23% of the phenotypic variation for plant height.

Epistatic interactions were identified for TCH as well (Table 25). Average variation explained by individual interactions was only 8%, and all three interactions combined accounted for 23% of the variation. In the combined analysis of the TCH, LG G seems to possess regions with great effect on TCH plant height. Epistatic loci 52 and 217 and QTL 178 were mapped on different genomic regions of chromosome G, which

suggests the importance of allelic variability within this chromosome on plant height phenotypic variation.

Table 25. RIL and TCH digenic epistatic interactions for plant height across environments.

	Locus			Class Means Loci A,B (cm)			
Env	A	B	R^2	430,430	430,7000	7000,430	7000,7000
RIL	111	235	0.08	109.55	120.00	113.46	109.75
	264	91	0.07	107.78	112.73	122.14	111.68
	267	97	0.08	116.55	105.23	113.56	115.89
	267	321	0.11	103.63	116.81	116.60	113.06
TCH	52	97	0.07	127.74	121.43	121.75	123.36
	111	275	0.08	121.81	125.68	126.97	122.39
	186	217	0.09	122.56	127.67	125.15	121.33

As mentioned before, the RIL did not segregate at any of the major dwarfing loci since both parental lines are three dwarf genotypes ($dw_1Dw_2dw_3dw_4$). Therefore, no QTL detection on proposed locations for Dw_2 (Lin et al., 1995) and Dw_3 (Klein et al., 2000) was expected. However, plant height QTL detected in LG A-b and LG B in this study are consistent with those reported by Pereira and Lee (1995), Tuinstra et al. (1996), and Rami et al. (1998); and all LG A-b QTL across RIL experiments mapped to the same genomic regions where Hart et al. (2001) reported a major plant height QTL. Hart et al. (2001) and Sanchez-Gomez (2002) also mentioned that variation in plant height may be

found among genotypes within a population derived from parental lines that possess the same allelic makeup at dwarfing loci. This strongly suggests the presence of modifying genes that are responsible for variation within same dwarf class genotypes.

It was somewhat surprising to not detect at least one QTL in common between RIL and TCH, since phenotypic correlation for plant height between populations was 0.53 (See Chapter III). While important QTL were mapped to LG A-b in RIL, the most consistent QTL in TCH was mapped to LG D-b. Also, ATx2752 is a three dwarf, thus no dominance gene action should act at the major loci in the testcrosses. Thus, it is very likely that dominance gene action at other heterozygous loci has a greater effect on plant height than what QTL A-b has.

Several authors have reported pleiotropic effects on yield components for genes involved in plant height. *Dw_3* has pleiotropic effect on kernels per panicle, kernel weight, tiller number and panicle size (Casady, 1965), and *Dw_2* may affect panicle length, main head yield, seed weight and leaf area (Graham and Lessman, 1966). Even though this RIL did not segregate for any of the dwarfing genes to which pleiotropism has been linked to, positive genetic correlations between GYL and PHE in both RIL and TCH suggests the presence of pleiotropic effects or linkage among loci involved in trait expression. QTL analyses does not eliminate the possibility of pleiotropic action of some genes, since plant height QTL 253 in RIL and QTL 178 and 284 in TCH were mapped to the exact same genomic regions as those QTL detected in the grain yield analysis. In the RIL, RTx7000 alleles have a positive effect on grain yield and plant height, while in TCH, RTx430 alleles increased yield and plant height.

Days to Mid-anthesis. High positive genetic correlations (0.67 – 0.99) among environments strongly suggested the possibility that the same genomic regions were involved in maturity variability across environments. QTL analyses of RIL and TCH revealed that two genomic regions were consistently involved in phenotypic variation of flowering behavior (Table 26). More importantly, such QTL were consistent across populations.

QTL 278 and 330 were mapped 7.1 cM apart in chromosome H, and QTL 54 and 245 were mapped 16.2 and 3.3 cM from QTL 273 in chromosome I (Figure 4). Assuming a confidence interval of 20 cM due to imprecise QTL peak location, it can be safely assumed that these QTL belong to the same genomic regions in chromosomes H and I, respectively. Positive additive effect of QTL in LG H results in delaying anthesis due to RTx430, while the negative effect of QTL 273 causes earliness due to RTx7000.

The QTL in LG I seem to be especially important since they account for as much as 27% of the PVE across RIL environments, while in TCH, they account for as much as 37%. QTL 278 was not detected in the CSH01 analysis, but QTL 273 was still detected. Unusual flowering behavior observed in TCH at this environment (Table 10) is likely responsible for the "lack of" significant effect of this genomic region on testcross behavior.

Table 26. Peaks, LOD, R^2, and additive genetic effect of days to mid-anthesis QTL in the RIL and TCH populations.

Env	LG	Peak Locus†	Position	LOD	R^2‡	Additive
CSI99	H	278	0.14	2.69	0.09	1.04
	I	**273**	**0.67**	**3.00**	**0.14**	**-1.33**
BEI99	B	163	0.58	2.85	0.07	0.98
	C	**261**	**0.04**	**3.12**	**0.09**	**-1.11**
	F	**294**	**1.54**	**3.07**	**0.08**	**1.09**
	G	36	0.99	2.43	0.05	0.88
	H	278	0.16	2.97	0.07	1.08
	H	**133**	**1.31**	**3.86**	**0.14**	**1.42**
	I	**273**	**0.71**	**4.45**	**0.14**	**-1.47**
CSI00	**B**	**57**	**0.61**	**4.99**	**0.12**	**0.84**
	D-b	81	0.89	2.31	0.05	0.53
	H	**330**	**0.17**	**4.29**	**0.09**	**0.78**
	H	333	1.46	2.93	0.07	0.62
	I	**54**	**0.46**	**3.74**	**0.10**	**-0.79**
RIL	**B**	**163**	**0.56**	**5.84**	**0.13**	**1.00**
Combined	D-b	301	0.99	2.34	0.06	0.65
	F	**294**	**1.54**	**3.71**	**0.09**	**0.83**
	H	**278**	**0.16**	**6.09**	**0.15**	**1.16**
	H	**133**	**1.33**	**2.83**	**0.08**	**0.78**
	I	**273**	**0.65**	**7.80**	**0.27**	**-1.51**
CSH00	B	309	0.80	2.25	0.04	0.48
	C	138	2.01	2.06	0.04	-0.48
	H	**278**	**0.16**	**5.42**	**0.13**	**0.90**
	I	**245**	**0.54**	**10.82**	**0.25**	**-1.33**
TAH00	**A-b**	**157**	**0.08**	**3.12**	**0.08**	**-0.52**
	H	**330**	**0.19**	**5.67**	**0.15**	**0.72**
	I	**245**	**0.52**	**3.97**	**0.09**	**-0.56**
	J	193	0.37	2.17	0.05	0.39
CSH01	**A-b**	100	0.41	2.78	0.09	-0.60
	D-a	329	0.15	2.59	0.05	-0.41
	I	**273**	**0.61**	**11.48**	**0.42**	**-1.23**
TCH	C	138	2.01	2.58	0.05	-0.38
Combined	**H**	**330**	**0.17**	**5.67**	**0.12**	**0.61**
	H	133	1.29	2.66	0.09	0.48
	I	**273**	**0.59**	**11.93**	**0.37**	**-1.14**

† Bold lettering indicates significant QTL according to permutation test LR.

‡ Denotes the percentage of phenotypic variation explained (PVE) by the locus.

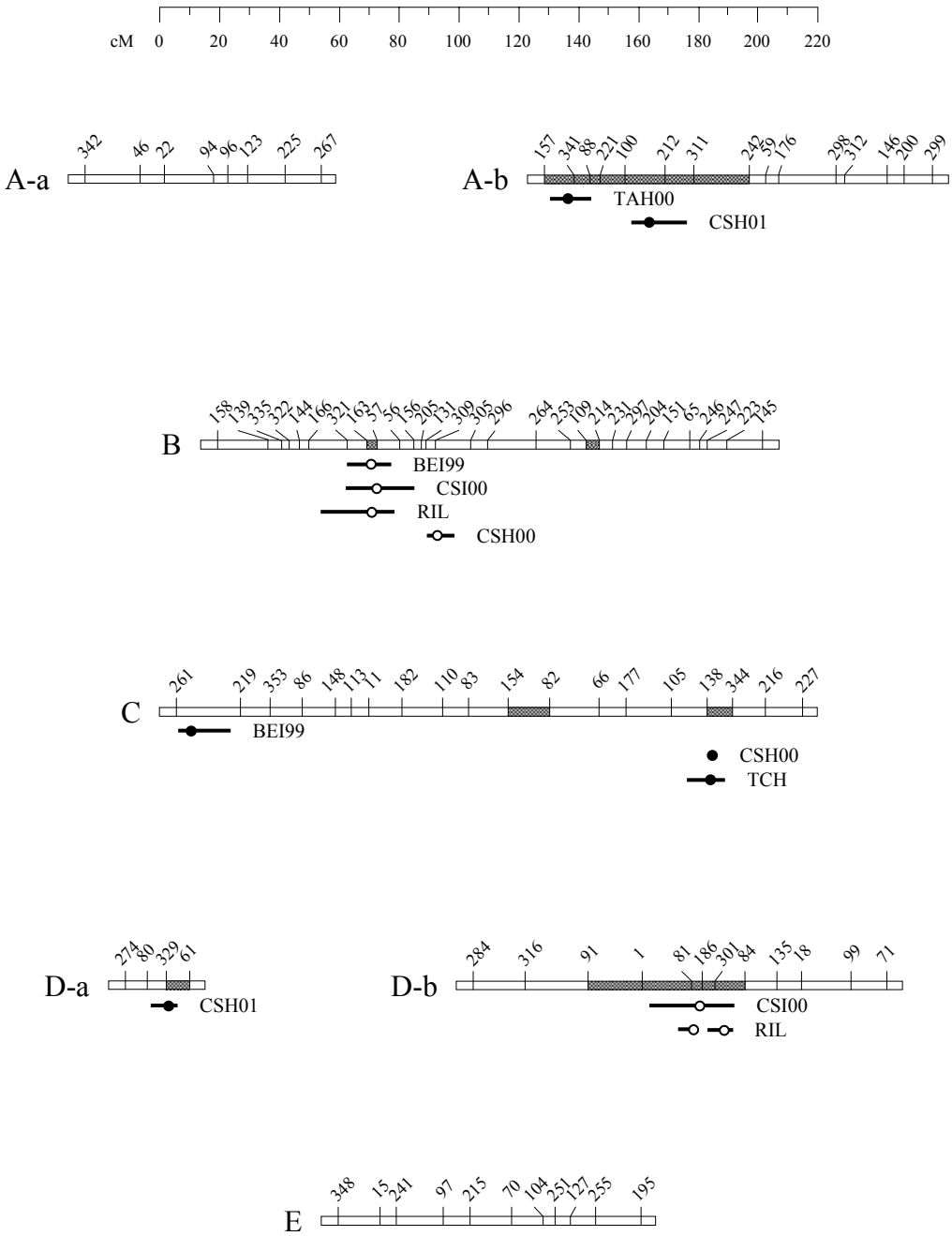

Figure 4. Putative days to mid-anthesis QTL at individual and across environments. Shaded regions of LG indicate segregation distortion. Bars show positions of QTL with the peak LOD-score identified with a circle. Open circles represent trait QTL affected by the RTx430 allele, while solid circles represent trait QTL affected by the RTx7000 allele.

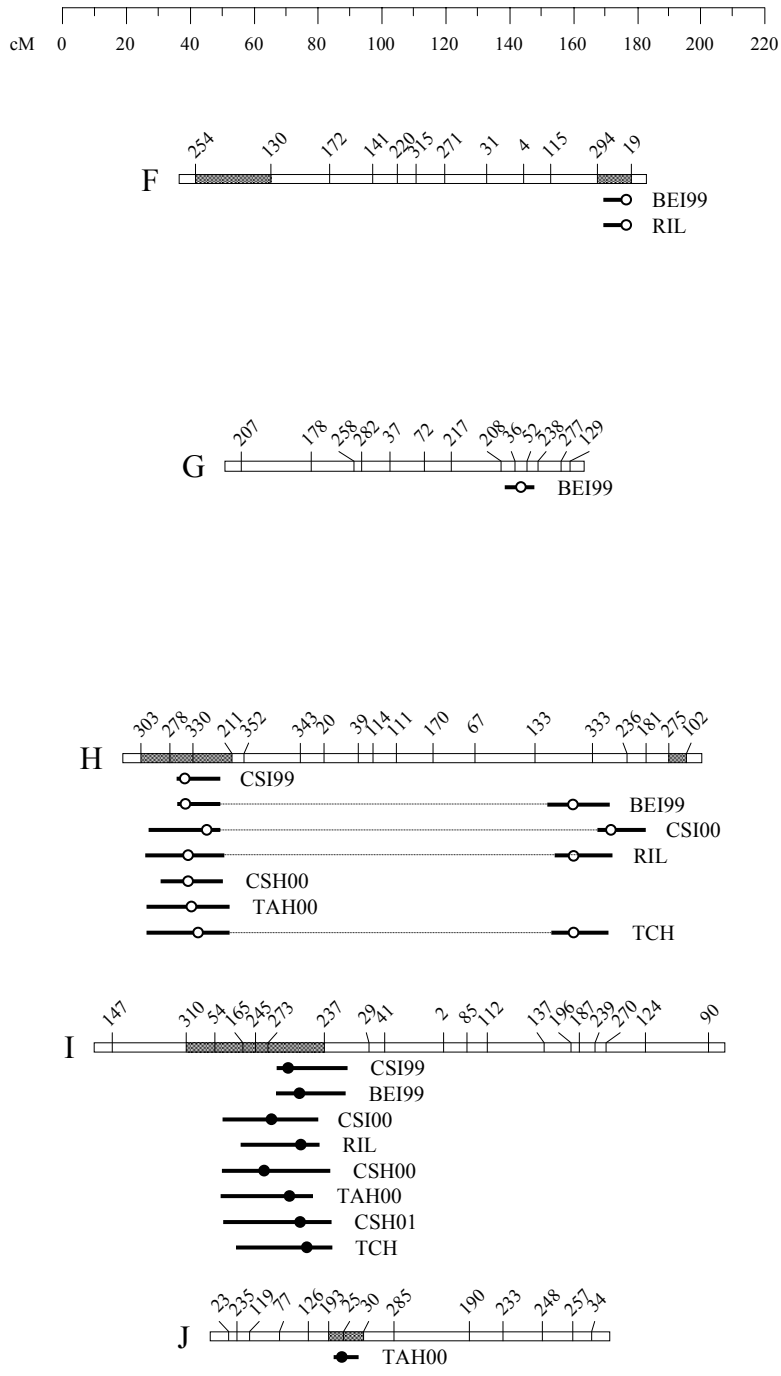

Figure 4. Continued.

With the exception of CSI99 and CSH00, possible colliniarity precluded the inclusion of all detected QTL in the full model, therefore one or two QTL were excluded from the analyses (See appendix). Combined QTL analysis of RIL at CSI99 explained 7.8% of the additive variation observed, 22.3% at CSI00, 14% at CI00, and as much as 29.9% across all RIL environments. Phenotypic variation explained by QTL was much higher in TCH, explaining as much as 30.7%, 19.3%, and 22.9% at CSH00, TAH00, and CSH01, respectively, while explaining 32.3% for the TCH combined analysis.

Fifteen and fourteen epistatic interactions were readily detected for RIL and TCH populations, respectively. However, only four could be included in the full model for RIL, whereas in TCH only two were included (Table 27). Variation explained by individual interactions ranged from 7% in TCH, to a high of 11% explained by loci 238 and 41 in RIL. Inclusion of interactions in the overall linear models greatly increased the fitness, but to a lesser extent than what was observed for YLD and PHE. Epistatic interactions alone explained 17.4% and 10.6% of PVE in RIL and TCH respectively, for an overall all factor model fitness of 58% in RIL and 48% in TCH.

Maturity locus mapped in LG B is probably located in the same genomic region where Tuinstra et al. (1996) and Crasta et al. (1999) reported a flowering QTL; and locus 294 was mapped in the distal section of chromosome G, where Kebede et al. (2001) reported another QTL. Nevertheless, without common anchor markers among linkage maps, it is impossible to determine if these regions are in fact the same.

Table 27. RIL and TCH digenic epistatic interactions for days to mid-anthesis across environments.

	Locus			Class Means Loci A,B† (days)			
Env	A	B	R^2	430,430	430,7000	7000,430	7000,7000
RIL	1	109	0.09	65.0	67.1	67.2	65.7
	126	91	0.10	68.8	65.6	65.5	66.2
	165	241	0.09	65.1	66.8	67.6	66.0
	238	41	0.11	66.9	65.8	65.3	67.7
TCH	11	196	0.10	74.6	74.3	73.4	75.2
	311	316	0.07	74.6	73.1	74.4	74.7

Ma_1 flowering gene is located in LG I (Lin et al., 1995), *Ma_3* is located in LG A (Childs et al., 1997), and *Ma_4* in LG G (Hart et al., 1991). Strong QTL (LOD > 3) detected suggests the presence of a segregating maturity gene in the LG I. However, *Ma_1* is specifically regulated by photoperiod (Quinby and Karper, 1945). While no definitive knowledge of the *$Ma_{1\text{-}6}$* genetics of the RIL parental lines is presented, it can be argued that RTx430 and RTx7000 react similarly to photoperiod variations, which strongly suggests that variation due to QTL 274 and 54 is not related to segregation of *Ma_1* across the RIL. Preliminary data indicate that *Ma_6* is located in LG I as well (P.E. Klein, TAMU, personal communication), but it is unlikely that this population is segregating for this maturity gene due to tight linkage between *Ma_1* and *Ma_6* loci. Until more knowledge is acquired on RTx430 and RTx7000 maturity genetics, nothing conclusive

may be said in regards to the mapped QTL in LG I and their possible relationship to maturity genes.

Correlations (Table 17 and 18) between DMA and PHE are not in agreement with the conclusion of Lin et al. (1995) that flowering is correlated to increased plant height in most Poaceae, suggesting the presence of either pleiotropic effects or different closely linked genes with independent effects on both traits. Quinby and Karper (1945) suggested that observed causal effect between flowering and plant height, might be due to closely linked Ma_1 and Dw_2 genes. Lack of consistent QTL detection across both traits in this study supports the linkage theory, as it has been demonstrated in wheat (Worland and Law, 1986). It is quite likely that lack of correlation observed between flowering and plant height is due to linkage breakage between loci involved in expression of both traits, which occurred during the development of this population.

Tillering Ability. Significant differences were detected in RIL tillering when CSI99 and BEI99 were compared, observing almost twice the number of tillers in College Station than in Beeville. A genomic region 18 cM from the top of LG A-a may to be responsible for tillering variation at both environments (Figure 5). At each environment where this QTL was detected, at least 12% of the phenotypic variability was accounted for by this genomic region on LG A-a. The RTx7000 alleles in QTL 46 and QTL 22 increased tillering ability of the genotypes at both environments. The same genomic region influenced tillering in the combined analysis as well, but with a smaller additive effect from RTx7000. Even though RTx430 was released as a parental line with increased capacity to produce basal tillers, especially under thin stands (Miller, 1984),

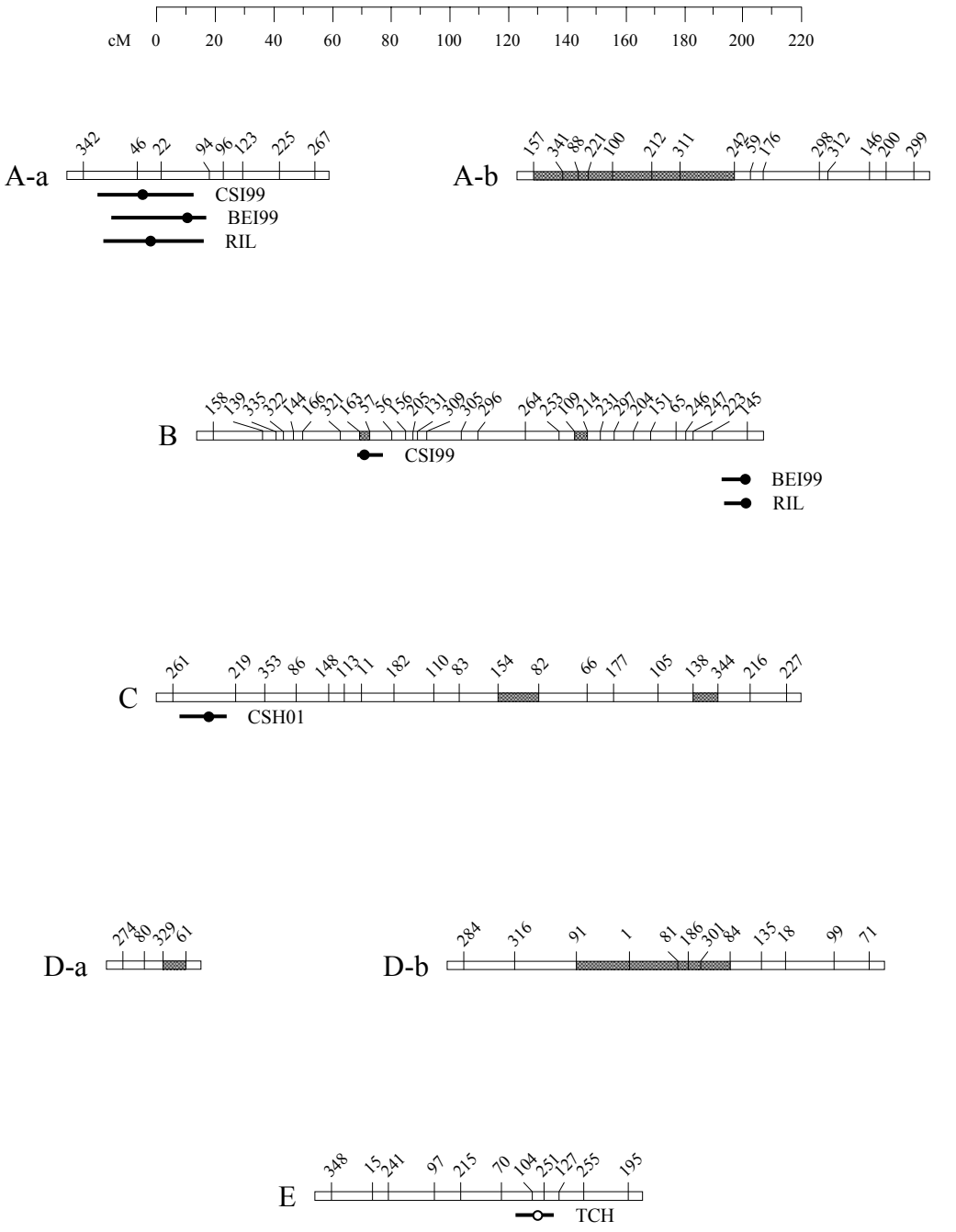

Figure 5. Putative tillering QTL at individual and across environments. Shaded regions of LG indicate segregation distortion. Bars show positions of QTL with the peak LOD-score identified with a circle. Open circles represent trait QTL affected by the RTx430 allele, while solid circles represent trait QTL affected by the RTx7000 allele.

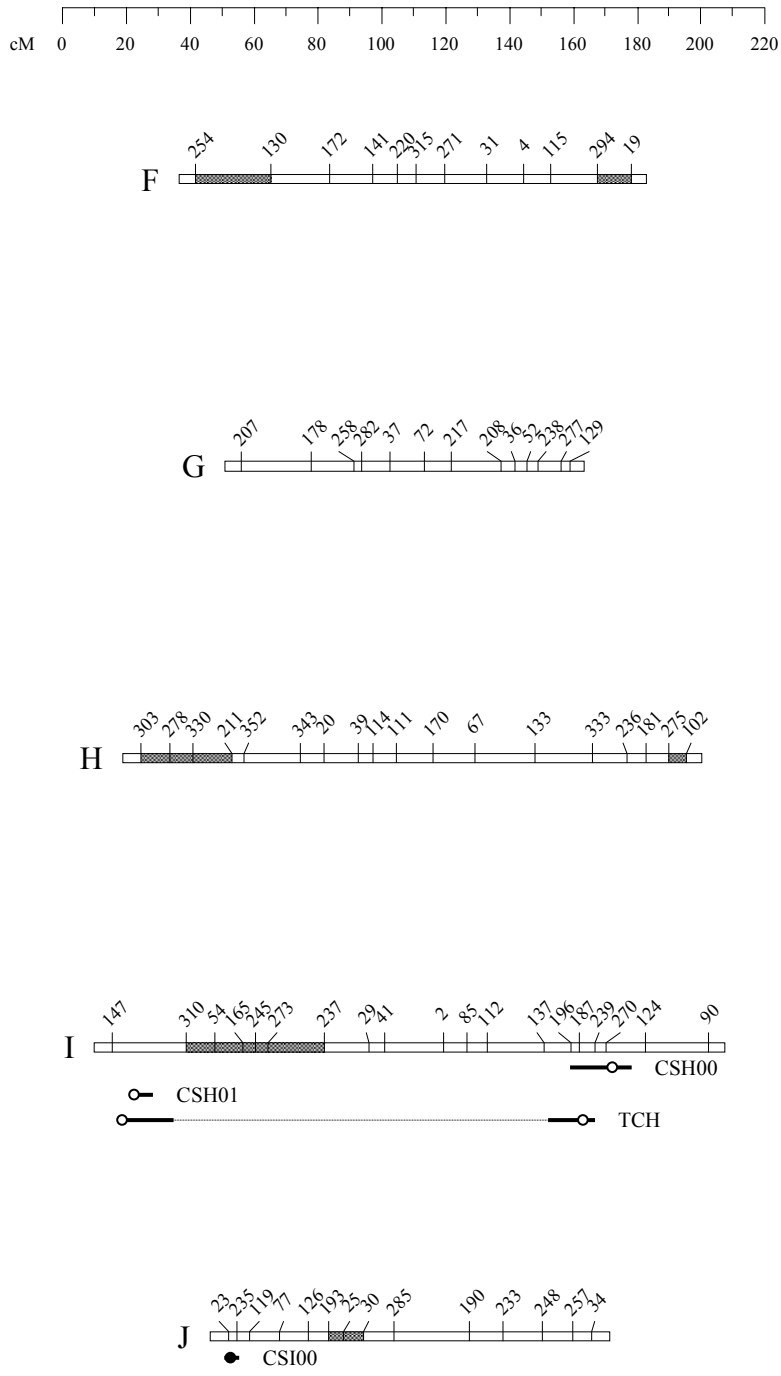

Figure 5. Continued.

RTx7000 has better tillering capacity. Additive models including putative QTL explained 12% of phenotypic variability observed in CSI99, 7% in BEI99, 5.6% in CSI00 and 10.6% across RIL environments (Table 28).

Even though QTL detected for TCH tillering were mapped in the same chromosome, they are located more than 50 cM apart. Thus none of the QTL were consistent across TCH environments. Alleles coming from RTx430 showed greater interaction with the ATx2752 alleles at each one of the QTL loci detected, increasing the tillering ability of the testcrosses (Table 28).

These results are in agreement with previous observations that grain yield in these populations is positively correlated with tillering ability of the genotype as well as with plant height. While selected genomic regions of RTx7000 had a positive effect on yielding capacity and plant height of inbred lines, alleles ascribable RTx430 increased grain yield and plant height in hybrid combination with ATx2752. As previously noted, the same can be observed for the effect of the parental lines on inbred per se tillering ability as well as in their testcrosses.

Six epistatic interactions were added to the putative QTL additive model to improve its fitness. Variation explained by each interaction for RIL varied between 8% and 12%; altogether they explained 29% (Table 29). The interaction of loci 154 and 352 explained more variation than that explained by QTL 223 in chromosome B. Genotypes that possessed RTx430 alleles in locus 154 and RTx7000 alleles in locus 352 yielded better than genotypes homozygous for either parental lines alleles at both loci. Based on this data, it appears that tillering is a quantitative trait that results from intra and

interallelic interaction among different loci. As it is shown, it is quite possible that interallelic interactions might be more important in quantitative trait expression than main QTL gene action. Thus, aiming for such loci through molecular breeding may certainly yield better results than concentrating on QTL dependant improvement.

Table 28. Peaks, LOD, R^2, and additive genetic effect of tillering QTL in the RIL and TCH populations.

Env	LG	Peak Locus†	Position	LOD	R^2‡	Additive
CSI99	**A-a**	**46**	**0.25**	**3.79**	**0.12**	**-1.55E4**
	B	163	0.58	2.75	0.07	-1.24E4
BEI99	**A-a**	**22**	**0.41**	**3.27**	**0.14**	**-1.04E4**
	B	**223**	**1.99**	**3.27**	**0.09**	**-8.41E3**
CSI00	**J**	**23**	**0.02**	**3.38**	**0.09**	**-8.03E3**
RIL	**A-a**	**46**	**0.27**	**4.10**	**0.13**	**-9.24E3**
Combined	B	223	1.97	2.60	0.08	-7.32E3
CSH00	**I**	**270**	**1.76**	**2.99**	**0.08**	**5.30E3**
CSH01	C	261	0.10	2.04	0.10	1.11E4
	I	147	0.00	2.18	0.06	7.92E3
TCH	E	104	0.76	2.45	0.06	4.77E3
Combined	I	147	0.00	2.69	0.06	5.02E3
	I	**187**	**1.67**	**3.64**	**0.09**	**6.14E3**

† Bold lettering indicates significant QTL according to permutation test LR.
‡ Denotes the percentage of phenotypic variation explained (PVE) by the locus.

Table 29. RIL and TCH digenic epistatic interactions for tillering across environments.

	Locus			Class Means Loci A,B† (units)			
Env	A	B	R^2	430,430	430,7000	7000,430	7000,7000
RIL	112	195	0.09	1.03E5	8.40E4	8.89E4	1.00E5
	126	216	0.10	8.06E4	1.01E5	1.00E5	8.74E4
	154	352	0.12	8.83E4	1.06E5	1.01E5	8.27E4
	195	36	0.08	1.05E5	9.08E4	8.35E4	9.95E4
	335	81	0.08	7.22E4	9.71E4	1.01E5	9.46E4
	41	65	0.10	7.89E4	1.06E5	9.74E4	9.22E4
TCH	148	214	0.09	1.35E5	1.44E5	1.49E5	1.34E5
	178	310	0.10	1.38E5	1.47E5	1.50E5	1.33E5
	18	267	0.08	1.45E5	1.36E5	1.33E5	1.47E5
	278	86	0.09	1.29E5	1.50E5	1.45E5	1.40E5

None of the tillering QTL identified in either population are located on the same chromosomes as those reported by Paterson et al. (1995), who identified four genomic regions that control seedling tillers in *Sorghum halapense* (L) Pers. However, Paterson et al. (1995) evaluated tillering in an interspecific cross with extreme variation for the characteristic. In the current study, the population represents a cross of elite inbred lines that display a much lower level of diversity for the trait. Hart et al. (2001) showed strong evidence for the presence of tillering QTL in chromosomes A and I. Although one tillering QTL for RIL was identified in chromosome A-a, it was determined by using anchor marker information that they are localized in different genomic regions.

Nevertheless, QTL 137 is located in the same genomic region in chromosome I where Hart et al. (2001) reported a tillering QTL.

Panicle Length. Several QTL were identified for panicle length in RIL and TCH (Figure 6). Although two or more QTL were identified as important within a chromosome by CIM, not all of them could be successfully added into the full linear additive model (See appendix), which implies certain degree of colliniarity among those QTL. This was somewhat surprising since CIM is designed to control for the presence of other QTL and identify the most significant QTL. Thus, model building should be used as a fail safe mechanism to correctly identify the QTL that in combination have the greatest effects on phenotypic variability.

Fitting of the full model by selected putative QTL for CSI99, BEI99, and CSI00 output a R^2 of 20.7%, 16.1% and 31.6%, respectively. Across environments, QTL explained as much as 31.6% of the panicle length variability. PVE by QTL in TCH across hybrids was less than expected, just 11.7%. Low trait heritabilities observed in the TCH environments might have limited capacity of the CIM analyses to identify panicle length QTL.

QTL 46 and QTL 212 were consistently identified in RIL 1999 environments, as well as across RIL environments (Table 30). The presence of the RTx430 allele in locus 46 consistently increased panicle length, while QTL 212 was associated with longer panicles due to RTx7000. In TCH, longer panicles were observed in genotypes that possessed RTx430 alleles at locus 333 at individual environments as well as in the combined analysis (Table 30). Genomic regions of LG G were consistently involved in

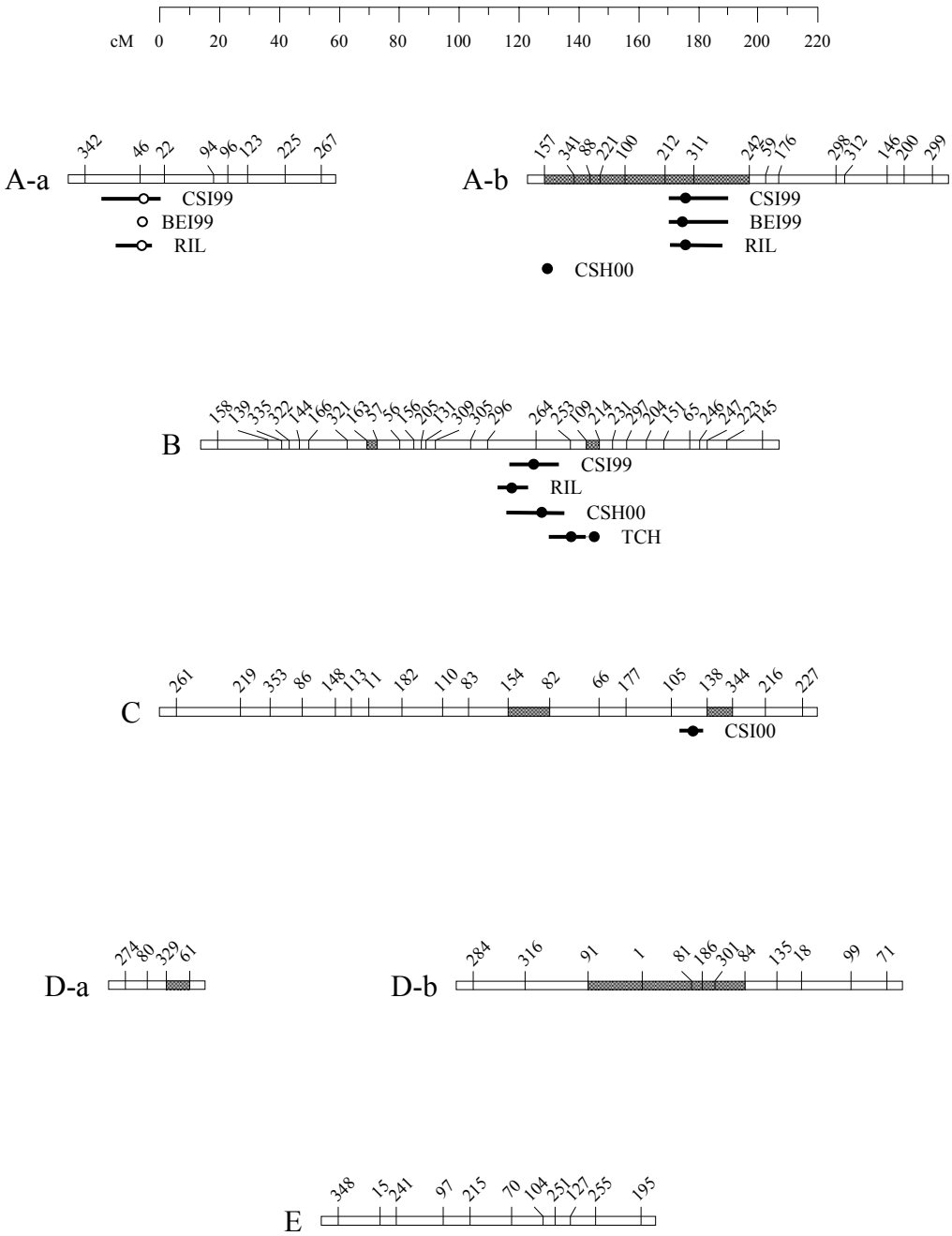

Figure 6. Putative panicle length QTL at individual and across environments. Shaded regions of LG indicate segregation distortion. Bars show positions of QTL with the peak LOD-score identified with a circle. Open circles represent trait QTL affected by the RTx430 allele, while solid circles represent trait QTL affected by the RTx7000 allele.

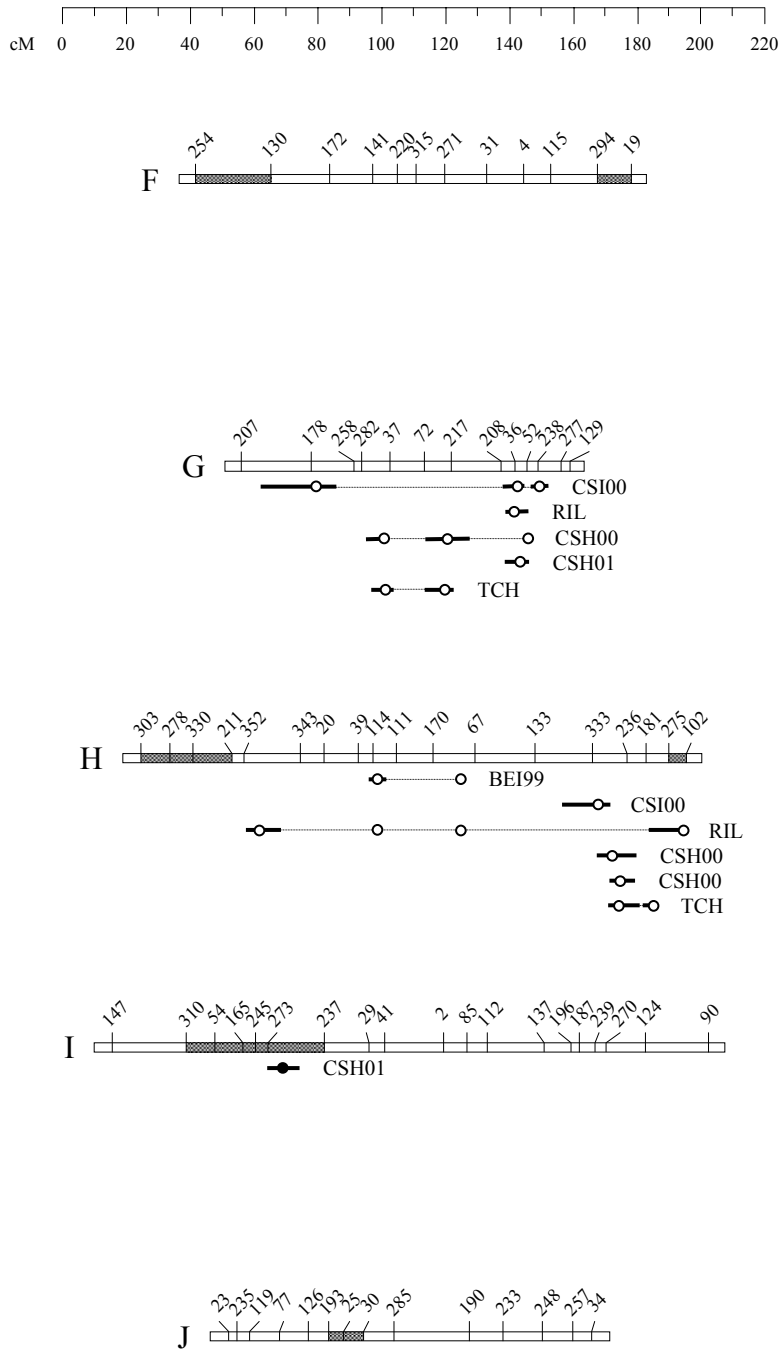

Figure 6. Continued.

Table 30. Peaks, LOD, R^2, and additive genetic effect of panicle length QTL in the RIL and TCH populations.

Env	LG	Peak Locus†	Position	LOD	R^2‡	Additive
CSI99	**A-a**	**46**	**0.21**	**5.56**	**0.14**	**0.99**
	A-b	**212**	**0.50**	**3.01**	**0.09**	**-0.86**
	B	296	1.13	2.33	0.06	-0.67
BEI99	A-a	46	0.21	2.10	0.05	0.64
	A-b	**212**	**0.50**	**3.16**	**0.10**	**-0.94**
	H	114	0.81	2.22	0.05	0.62
	H	170	1.15	2.38	0.06	0.65
CSI00	C	105	1.95	2.27	0.07	-0.79
	G	178	0.26	2.63	0.06	0.74
	G	36	0.97	3.78	0.08	0.89
	G	238	1.05	2.56	0.06	0.73
	H	**333**	**1.38**	**4.06**	**0.09**	**0.91**
RIL	**A-a**	**46**	**0.21**	**3.95**	**0.08**	**0.69**
Combined	A-b	212	0.52	2.77	0.07	-0.67
	B	296	1.07	2.38	0.07	-0.59
	G	**36**	**0.97**	**3.39**	**0.07**	**0.63**
	H	352	0.39	2.26	0.05	0.53
	H	114	0.81	2.00	0.04	0.44
	H	170	1.15	2.08	0.04	0.47
	H	275	1.68	2.77	0.06	0.56
CSH00	A-b	157	0.00	2.02	0.04	-0.57
	B	264	1.23	2.84	0.08	-0.69
	G	**282**	**0.54**	**3.99**	**0.09**	**0.92**
	G	**72**	**0.74**	**3.78**	**0.10**	**-1.02**
	G	52	1.01	2.03	0.05	0.65
	H	333	1.44	3.01	0.09	0.74
CSH01	**G**	**36**	**0.97**	**3.30**	**0.08**	**0.58**
	H	333	1.48	2.26	0.06	0.50
	I	273	0.59	2.26	0.08	-0.66
TCH	B	264	1.29	2.43	0.05	-0.41
	B	109	1.40	2.63	0.06	-0.44
	G	**282**	**0.54**	**3.72**	**0.09**	**0.66**
	G	**72**	**0.72**	**4.49**	**0.13**	**-0.87**
	H	333	1.46	2.77	0.08	0.50

† Bold lettering indicates significant QTL according to permutation test LR.
‡ Denotes the percentage of phenotypic variation explained (PVE) by the locus.

panicle length variation at individual TCH environments as well as across environments. However, specific QTL in LG G were not consistent across environments or in the combined environments.

Epistasis accounted for 12.6% of the variability observed among RIL, and 21% for TCH (Table 31). Several of the epistatic loci were mapped to LG C, which suggests that genomic regions in this chromosome may also play an important role in trait expression. These interactions did not explain more than 10% of the PVE. In RIL, longer panicles were consistently observed in genotypes with RTx430 alleles at locus 277 and RTx7000 alleles at locus 301, averaging a total length of approximately 29 cm. In this specific case, the positive effect comes from the substitution of RTx7000 for RTx430 alleles at locus 277 and its interaction with a homozygous locus 301 for RTx7000. For TCH, longer panicles were generally observed in genotypes with a combination of RTx7000 alleles at locus A, and RTx430 alleles at locus B (Table 31).

Rami et al. (1998) and Hart et al. (2001) mapped panicle length QTL on six and three chromosomes, respectively. On the basis of map positions, only QTL mapped by Rami et al. (1998) in LG A and LG B could potentially be similar to QTL (QTL 46 and 212) mapped in the present study. Even though consistency in identification of QTL 333 in LG H across TCH environments might make it a candidate for being the same as the one reported by Hart et al. (2001), by anchor marker utilization it was determined that both QTL might be actually located in different chromosomal arms.

Table 31. RIL and TCH digenic epistatic interactions for panicle length across environments.

	Locus			Class Means Loci A,B (cm)			
Env	A	B	R^2	430,430	430,7000	7000,430	7000,7000
RIL	11	215	0.08	28.78	27.64	27.52	28.98
	138	145	0.08	27.61	28.30	29.45	27.35
	277	301	0.07	27.63	29.35	28.13	27.37
TCH	110	303	0.08	26.47	26.90	27.82	26.13
	148	316	0.09	26.35	27.16	27.27	25.86
	193	37	0.10	26.66	27.10	27.53	25.62
	248	80	0.08	27.44	26.59	25.99	27.07
	34	344	0.07	26.33	27.67	26.78	26.15

Panicle Exsertion. At least three QTL were identified across all experiments, with a wide range of phenotypic variance explained by each (Figure 7). The genomic region at 120 cM in LG H seems to encompass a major gene(s) responsible for panicle exsertion in the RIL, since QTL 133 was consistently identified at each individual environment as well as across environments. This QTL by itself explained as much as 30% of the PVE observed in RIL at BEI99, and as much as 32% in the combined analysis. Among QTL identified for all agronomic traits examined in this population, this QTL has explained the most variability. Two other QTL in the combined analysis, QTL 247 in LG B and QTL 178 in LG G, were determined to be highly significant by the permutation test (Table 32). With the exception of CSI00, for all RIL experiments

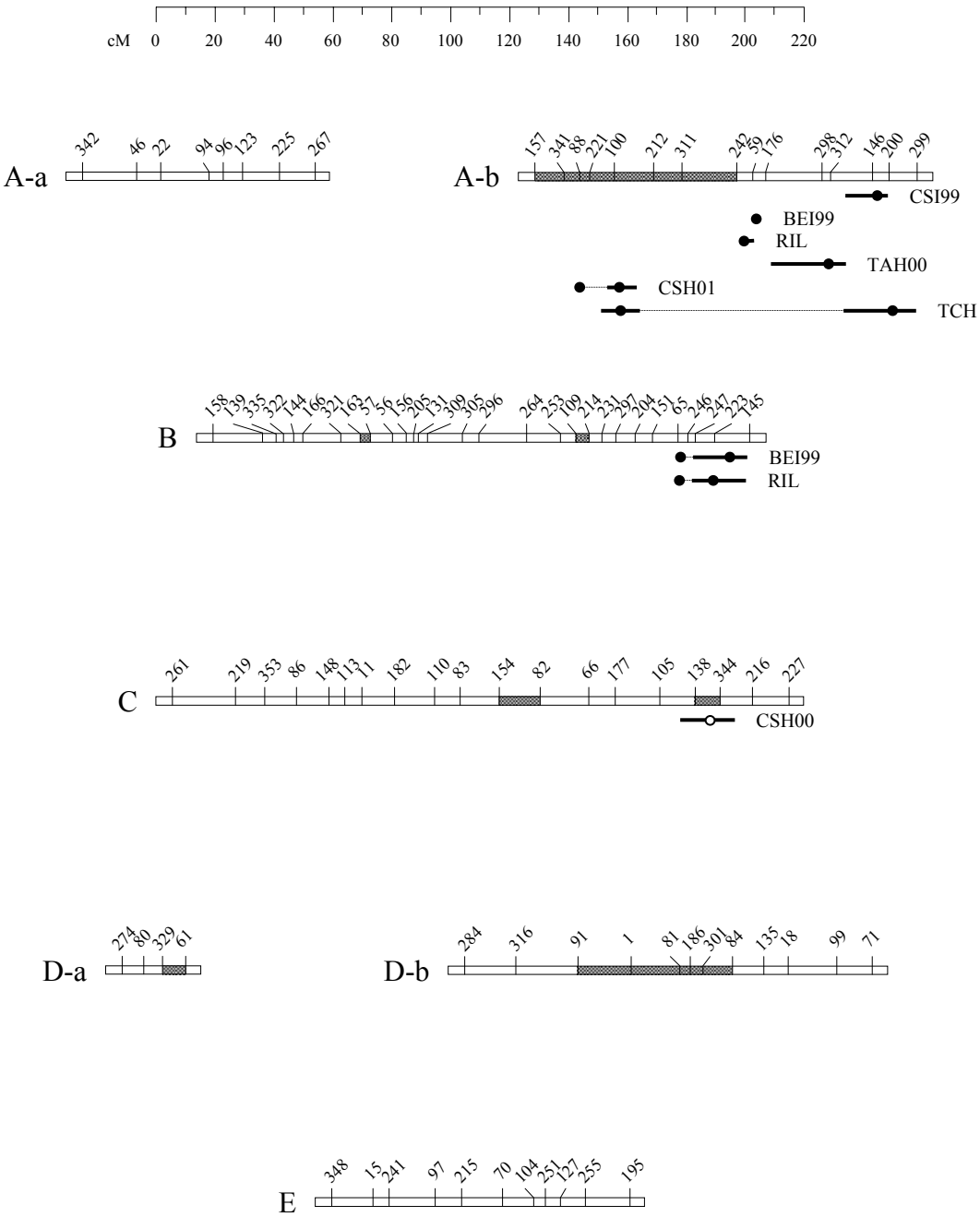

Figure 7. Putative panicle exsertion QTL at individual and across environments. Shaded regions of LG indicate segregation distortion. Bars show positions of QTL with the peak LOD-score identified with a circle. Open circles represent trait QTL affected by the RTx430 allele, while solid circles represent trait QTL affected by the RTx7000 allele.

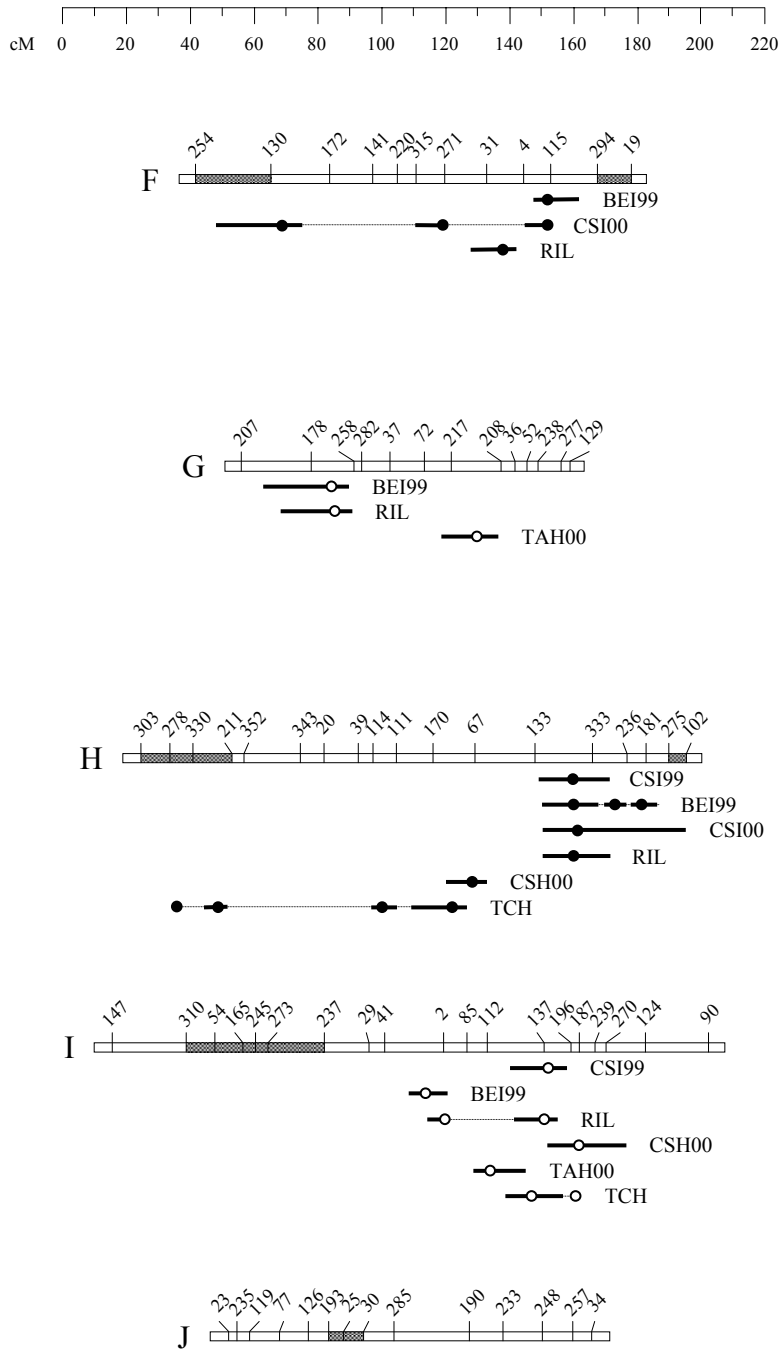

Figure 7. Continued.

Table 32. Peaks, LOD, R^2, and additive genetic effect of panicle exsertion QTL in the RIL and TCH populations.

Env	LG	Peak Locus†	Position	LOD	R^2‡	Additive
CSI99	A-b	146	1.31	2.36	0.06	-1.26
	H	**133**	**1.27**	**4.23**	**0.15**	**-1.94**
	I	137	1.56	2.84	0.07	1.34
BEI99	A-b	59	0.82	2.16	0.04	-1.04
	B	**223**	**1.89**	**4.37**	**0.12**	**-1.71**
	F	4	1.25	2.60	0.06	-1.24
	G	**178**	**0.36**	**3.72**	**0.11**	**1.65**
	H	**133**	**1.27**	**8.98**	**0.30**	**-2.67**
CSI00	F	130	0.34	2.67	0.11	-1.33
	F	271	1.00	2.37	0.08	-1.16
	H	**133**	**1.33**	**4.68**	**0.17**	**-1.68**
RIL	A-b	242	0.81	2.15	0.05	-0.89
Combined	**B**	**247**	**1.83**	**3.83**	**0.10**	**-1.28**
	F	31	1.09	2.53	0.07	-1.07
	G	**178**	**0.34**	**3.45**	**0.10**	**1.27**
	H	**133**	**1.29**	**8.73**	**0.32**	**-2.22**
	I	2	1.19	2.21	0.04	0.84
CSH00	C	138	2.07	2.51	0.07	1.02
	H	**170**	**1.13**	**2.87**	**0.08**	**-1.13**
	I	**187**	**1.70**	**4.72**	**0.12**	**1.36**
TAH00	A-b	312	1.13	2.80	0.07	-0.70
	G	**217**	**0.79**	**4.63**	**0.14**	**1.05**
	I	112	1.34	2.47	0.06	0.66
CSH01	A-b	100	0.29	2.74	0.07	-1.54
TCH	A-b	100	0.29	2.81	0.07	-0.94
Combined	A-b	200	1.35	3.05	0.07	-0.69
	B	297	1.50	2.17	0.05	0.62
	H	330	0.25	2.33	0.07	-0.73
	H	**170**	**1.05**	**3.11**	**0.09**	**-0.79**
	I	112	1.48	2.98	0.11	0.84

† Bold lettering indicates significant QTL according to permutation test LR.
‡ Denotes the percentage of phenotypic variation explained (PVE) by the locus.

including the combined analysis, the putative QTL model explained at least 20% of the panicle variability observed among RIL (See appendix).

Even though several panicle exsertion QTL for TCH were mapped in the same LG as those mapped for RIL, only those in LG I are close enough (within a 20 cM distance) to be considered the same. The only permutation-significant TCH QTL (QTL 112, LOD = 3.11) in LG I was mapped 31.7 cM apart from RIL QTL 133. Because of this distance, it is highly improbable that both genomic regions are actually the same.

Many epistatic interactions with effect on panicle exsertion were identified for RIL. However, none could be added to the putative QTL model. As previously mentioned, the presence of strong QTL might have shadowed the minor effect on interactions, thus precluding their inclusion in the full model.

For TCH, the interaction of loci 247 and 312 explained 9% of the trait variability. The longest exsertion occurs in genotypes that possess at least one allele from RTx430 at locus 247, while having at least one RTx7000 allele at locus 312. The shortest exsertion is observed in genotypes carrying RTx430 alleles at both loci.

Klein et al. (2001) reported six genomic regions responsible for variation in panicle exsertion in a population derived from RTx430 and Sureño. None of the QTL identified mapped to the same chromosomes, with the exception of QTL 242. However, this QTL is not within the confidence interval reported by the authors. Sanchez-Gomez (2002) also has identified two QTL responsible for exsertion in sorghum, but such QTL

are not located in the same genomic regions responsible for variation in this RIL population.

Panicle Weight. No QTL were identified for this trait in CSI99 and BEI99, although several were identified in CSI00 (Figure 8). Close examination of genetic variability at each environment revealed that genetic variance among RIL in CSI00 was 300% larger than when the inbreds were evaluated in the other two environments. Inability to properly capture variability due to genetic factors negatively affected the capability of the analysis to effectively detect QTL in the 1999 environments. Due to this problem, it may be better not to consider QTL across RIL environments, but only those from the CSI00 environment.

The only QTL that was statistically significant (LOD = 2.83) according to the permutation was QTL 297, which explained 8% of the variability observed among the inbreds in this environment (Table 33). When QTL loci (LOD > 2) were combined into one analysis, they explained as much as 16.7% of the PVE. With the exception of QTL 181, increased panicle weight QTL was correlated to the presence of RTx7000 alleles.

Several QTL were identified across TCH experiments, but none were consistent across environments. PVE by the putative QTL model was 9% in CSH01, 7% in TAH00, and 10% in CSH01. Across TCH environments, the fitness of the model was greatly improved by the inclusion of epistatic factors, since QTL per se explained 9.4% of the variability observed, while the QTL and epistasis model explained as much as 32.7%.

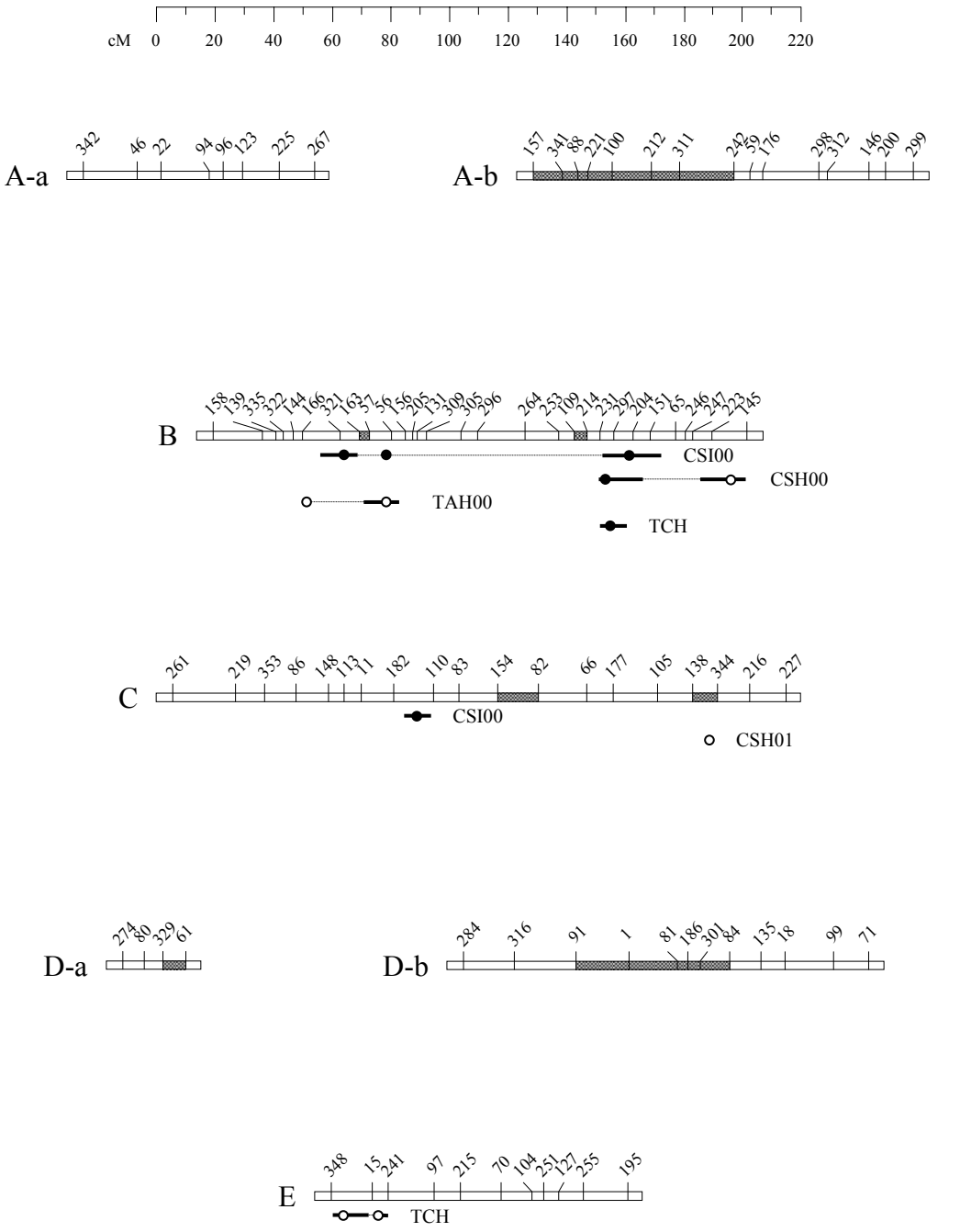

Figure 8. Putative panicle weight QTL at individual and across environments. Shaded regions of LG indicate segregation distortion. Bars show positions of QTL with the peak LOD-score identified with a circle. Open circles represent trait QTL affected by the RTx430 allele, while solid circles represent trait QTL affected by the RTx7000 allele.

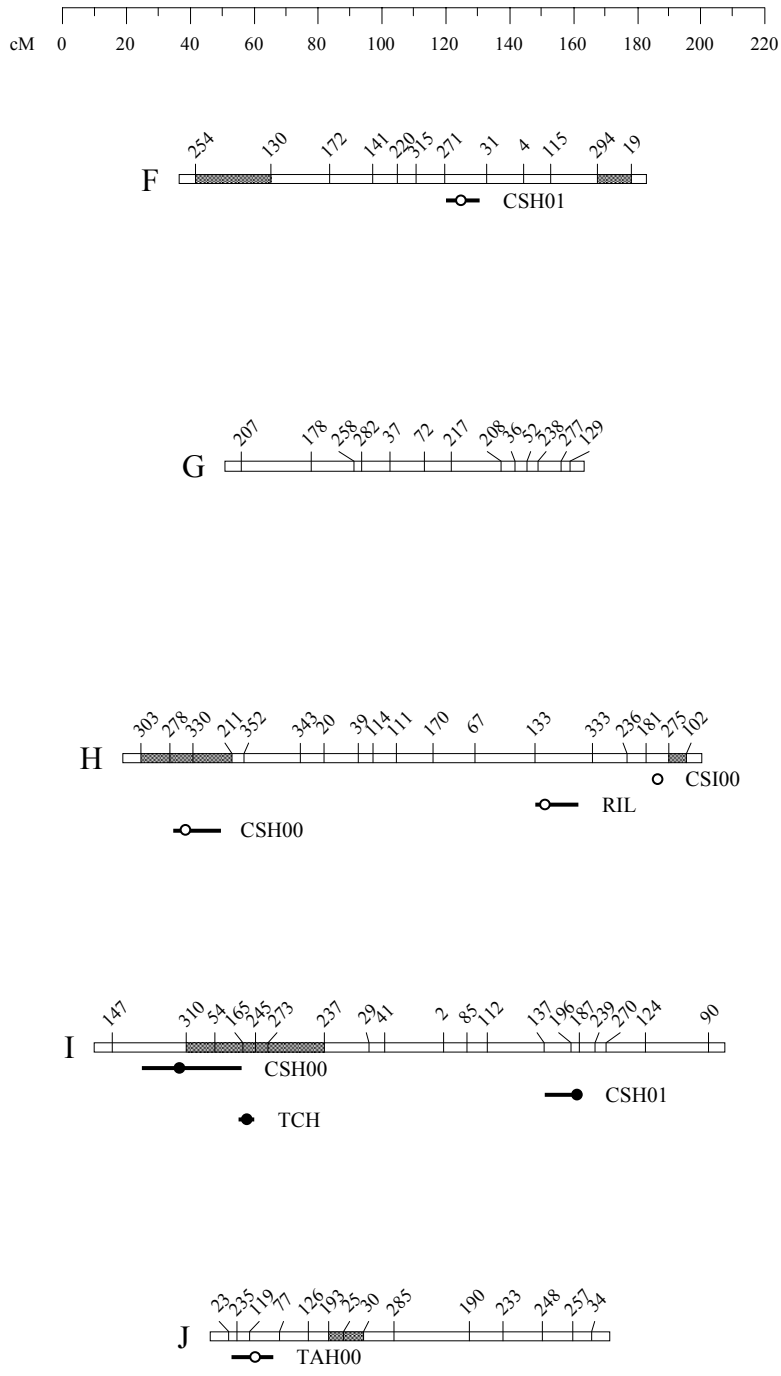

Figure 8. Continued.

Table 33. Peaks, LOD, R^2, and additive genetic effect of panicle weight QTL in the RIL and TCH populations.

Env	LG	Peak Locus†	Position	LOD	R^2‡	Additive
CSI00	B	321	0.48	2.42	0.06	-2.80
	B	57	0.63	2.03	0.06	-2.82
	B	**297**	**1.54**	**2.83**	**0.08**	**-3.30**
	C	182	0.95	2.07	0.07	-3.03
	H	181	1.58	2.01	0.06	2.76
RIL Combined	H	133	1.21	2.78	0.09	1.97
CSH00	**B**	**231**	**1.48**	**4.23**	**0.12**	**-3.06**
	B	**223**	**1.93**	**3.12**	**0.09**	**2.67**
	H	278	0.14	2.67	0.07	2.48
	I	**147**	**0.26**	**4.45**	**0.13**	**-3.12**
TAH00	**B**	**57**	**0.63**	**3.13**	**0.10**	**-2.15**
	J	**119**	**0.08**	**3.38**	**0.10**	**1.95**
CSH01	F	271	0.94	2.48	0.08	1.18
	I	196	1.67	2.84	0.08	-1.17
TCH Combined	B	231	1.48	2.88	0.09	-1.35
	E	348	0.08	2.52	0.09	1.17
	I	165	0.47	2.11	0.06	-1.03

† Bold lettering indicates significant QTL according to permutation test LR.
‡ Denotes the percentage of phenotypic variation explained (PVE) by the locus.

Several epistatic interactions with affecting TCH panicle weight were identified. Higher weights are observed with RTx430 alleles at loci 146 and 235 and RTx7000 alleles at loci 2 and 255 (Table 34). Even better panicle weights are seen when RTx7000 interact with ATx2752 interact at loci 311 and 80.

Analyses on phenotypic data from this study revealed positive correlations among grain yield, panicle weight, and plant height. However, molecular marker genotyping did not reveal an association between panicle weight and the other two traits. None of the genomic regions responsible for variation in panicle weight were similar to genomic regions associated with grain yield and plant height. For these two traits, positive effects were generally associated with RTx430 while no definitive pattern may be observed for panicle weight. Either parental line may increase panicle weight at different loci. Nevertheless, a common QTL was found between panicle length and weight. Panicle length QTL 109 in LG B is located 8.4 apart from QTL 231, which has a significant effect on panicle weight variation among TCH. At both loci, RTx7000 increased the length and the weight of the panicle.

Table 34. RIL and TCH digenic epistatic interactions for panicle weight across environments.

	Locus			Class Means Loci A,B (g)			
Env	A	B	R^2	430,430	430,7000	7000,430	7000,7000
RIL	109	165	0.08	28.90	32.05	33.77	28.96
	219	72	0.08	28.94	31.40	35.62	30.20
	251	37	0.08	28.77	31.70	34.25	29.73
	267	278	0.09	28.39	32.42	34.41	29.44
	309	61	0.11	29.43	32.59	33.94	27.83
TCH	146	2	0.08	34.04	37.61	35.71	34.64
	235	255	0.08	34.60	37.91	35.77	34.35
	311	80	0.11	36.43	32.77	34.92	36.71

Conclusions

Numerous QTL were identified for each individual trait for RIL and TCH, but most of these QTL were not consistent across environments. Significant genotypes by environment interactions, detected during the phenotypic analysis of the traits, are a possible cause of the inconsistency. Nevertheless, a few key QTL were identified and the source of the positive additive genetics isolated.

Although high heritabilities for RIL were observed for grain yield at individual environments, the number of QTL detected were extremely low. Efficiency of QTL detection depends on several factors including type of molecular marker, QTL identification methodology, mapping population, marker density, sample size, and trait heritability (Ajmone Marsan et al., 2001). Simulations studies have indicated that the population size and heritability are most influential factors on the QTL detection power (Beavis, 1994). Although chromosome gaps are found in LG A and LG D, the coverage of the linkage map is quite good, averaging less than 10 cM between markers. With the exception of few outcrosses that were eventually eliminated, the utilization of dominant type marker and an advanced generation recombinant inbred population should have precluded the calling of false positives. Thus, there are two reasons for the low power of QTL detection in this specific study. First, the lack of grain yield QTL detection might have been due to a small population size. However, to consider population sizes larger than the one utilized in this study is highly impractical and rather costly to be even

considered; and secondly, it is quite possible that the effect of individual QTL is smaller than the overall experimental error rate.

QTL detected for grain yield and maturity further verified the existence of a tight relationship between both traits, since the presence of pleiotropic effect was identified of one genomic region in RIL and two in TCH on plant height and grain yield. In RIL, the identified QTL consistently increased grain yield and plant height due to the presence of RTx7000 alleles, while in testcrosses, RTx430 positively affected both traits. This is strong evidence of what it has been long known, that RTx430 while a formidable parental line in hybrid combination, lacks performance as a line per se.

Lastly, specific genomic regions from RTx7000 may be utilized to improve the performance of RTx430 as a line se. However, none of the recombinant inbred lines was a better parental line than RTx430 in hybrid combination. Undoubtedly RTx430 carries such an excellent genetic constitution that any attempts to derive a newer better inbred line from a cross of RTx430 and RTx7000, results in disruption of elite genetic blocks that are crucial for enhanced combining ability of RTx430.

CHAPTER V

SUMMARY

A mapping population composed of 187 $F_{5:6}$ recombinant inbred lines (RIL) was derived from the cross of restorer lines RTx430 x RTx7000. Because performance in hybrid combination is more important than the performance of the inbred line per se, a testcross hybrid population was developed by using each RIL as a pollinator on ATx2752. The RIL per se (RIL) and testcross hybrids (TCH) were evaluated at a total of three environments at two locations. Seven phenotypic characters, including grain yield, plant height, days to mid-anthesis, panicle number, panicle length, panicle exsertion and weight, were measured in both populations (RIL and TCH) at all environments. Heritability was estimated for each of the traits at each individual environment and across environments; and genotypic and phenotypic correlations were calculated among these traits as well.

Variation was detected among lines in the recombinant line population for all traits examined. Superior transgressive segregants were identified for each trait in the RIL; yet, when these superior genotypes were testcrossed, the resulting hybrids were not statistically better performers than the best parental testcross. Reductions in genetic variation were observed in harsher environments including Beeville, Texas, where severe drought persisted. However, the highly positive GYL correlation (r_G = 0.72)

observed between stress and non-stress environments suggests that the selection of superior genotypes may be done at either location. Causal relationships were detected between traits evaluated. Definitively, taller RIL tended to yield more as lines per se and as hybrids than shorter RIL. A positive correlation between the height of RIL and TCH may allow for an indirect selection for higher yield in hybrid combination by selecting taller genotypes during the breeding process.

AFLP and SSR primer combinations generated 354 polymorphic markers in the restorer RIL population with a subset of 174 marker loci being utilized to construct the linkage map. Loci were assigned to 12 different linkage groups. The 12 linkage groups were successfully assigned to the 10 sorghum chromosomes by means of anchor marker information. This linkage map covers 1573 cM with marker loci spaced at an averaged 9.04 cM, with interval distances ranging from 2.1 to 22.8 cM.

Numerous QTL were identified for each individual trait for RIL and TCH, but many of these QTL were not consistent across environments. Significant genotype by environment interactions, detected during the phenotypic analysis of the traits, likely had great effect on this inconsistency. Nevertheless, a few key QTL were identified and the source of the positive additive genetics isolated.

Close association between grain yield and maturity was identified in one genomic region in RIL and two in TCH. In RIL, the identified QTL consistently increased grain yield and plant height due to the presence of RTx7000 alleles, while in testcrosses, RTx430 positively affected both traits. Some genomic regions from RTx7000 may be utilized to improve RTx430 as a line se. However, it is very unlikely

that such regions will have a positive effect on the combining ability of RTx430 since testcross results did not reveal any transgressive segregants from the RIL population.

REFERENCES

Agmone, H.A., G.E. Wilde, J.C. Reese, L.R. Campbell, and M.R. Tuinstra. 2002. Genetic mapping of QTLs associated with greenbug resistance and tolerance in *Sorghum bicolor*. Theor. Appl. Genet. 104:1373-1378.

Ajmone Marsan, P., C. Gorni, A. Chittò, R. Redaelli, R. van Vijk, P. Stam, and M. Motto. 2001. Identification of QTLs for grain yield and grain-related traits of maize (*Zea mays* L.) using and AFLP map, different testers, and cofactor analysis. Theor. Appl. Genet. 102:230-243.

Basten, C.J., B.C. Weir, and Z.B. Zeng. 2002. QTL cartographer: A reference manual and tutorial for QTL mapping. Department of Statistics. NCSU, Raleigh.

Beavis, B. 1994. The power and deceit of QTL experiments: Lessons from comparative QTL studies. p. 250-266. *In* D.B. Wilkinson (ed.) Proc 49th Ann. Corn and Sorghum Industry Res. Conf. Chicago, Illinois. December 7-8. ASTA. Washington, D.C.

Bernardo, R. 1992. Relationship between single-cross performance and molecular marker heterozygosity. Theor. Appl. Genet. 83:628-634.

Brondani, C., P.H.N. Rangel, R.P.V. Brondani, and M.E. Ferreira. 2002. QTL mapping and introgression of yield-related traits from *Oriza glumaepatula* to cultivated rice (*Oriza sativa*) using microsatellite markers. Theor. Appl. Genet. 104:1192-1203.

Bubeck, D.M., M.M. Goodman, W.D. Beavis, and D. Grant. 1993. Quantitative trait loci controlling resistance to gray leaf spot in maize. Crop Sci. 33:838-847.

Cardinal A.J., M. Lee, N. Sharopova, W.L. Woodman, and M.J. Long. 2001. Genetic mapping and analysis of quantitative trait loci in maize for resistance to stalk tunneling by the European corn borer. Crop Sci. 41:835-845.

Casady, A.J. 1965. Effect of single height (*Dw*) gene of sorghum on grain yield components and test weight. Crop Sci. 5:385-389.

Chittenden, L.M., K.F. Schertzm Y.-R. Lin, R.A. Wing, and A.H. Paterson. 1994. A detailed RFLP map of *Sorghum bicolor x S. propinquum*, suitable for high density mapping, suggests ancestral duplication of sorghum chromosomes or chromosomal segments. Theor. Appl. Genet. 87:925-933.

Comstock, R.E., and H.F. Robinson. 1952. Genetic parameters, their estimation and significance. p. 284-291. *In* Wagner et al. (ed.) Proc. 6th Intern. Grassland Congr. State College, Pennsylvania. August 17-23.

Conner, A.B., and R.E. Karper. 1927. Hybrid vigor in sorghum. Texas Agri. Exp. Sta. Bul. 359.

Chung, J.H., and G.L.H. Liang. 1970. Some biometrical studies on nine agronomic traits in grain sorghum, *Sorghum bicolor* (L.) Moench. I. Variance components and heritability estimates. Can. J. Genet. Cytol. 12:288-296.

Churchill, G.A., and R.W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138:963-971.

Crasta, O.R., W.W. Xu, D.T. Rosenow, J. Mullet, and H.T. Nguyen. 1999. Mapping of post-flowering drought resistance traits in grain sorghum: Association between QTLs influencing premature senescence and maturity. Mol. Gen. Genet. 262:579-588.

Dalton, L.G. 1967. A positive regression of yield on maturity in sorghum. Crop Sci. 7:271.

Doerge, R.W., and G.A. Churchill. 1996. Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285-294.

Dogget, H. 1998. Sorghum. 2nd ed. Wiley, New York.

Dudley, J.W., M.A. Saghai Maroof, and G.K. Rufener. 1991. Molecular markers and grouping of parents in maize breeding programs. Crop Sci. 31:718-723.

Dudley, J.W. 1993. Molecular markers in plant improvement: Manipulation of genes affecting quantitative traits. Crop Sci. 33:660-668.

Dufour, P. 1996. Cartographie moléculaire du genome du sorgho (*Sorghum bicolor* L. Moench): Application on selection variétale; cartographie compare chez les andropogonees. Ph.D. dissertation. Université de Paris Sud. France.

Edwards, M.D., C.W. Stuber, and J.F. Wendel. 1987. Molecular marker-facilitated investigations of quantitative trait loci in maize. I. Numbers, distributions, and types of gene action. Genetics 116:113-125.

Fehr, W.R. 1991. Principles of cultivar development. Volume 1. Theory and technique. Iowa State University, Ames.

Freyer, R., and D.S. Douches. 1994. Development of a model for marker-assisted selection of specific gravity in diploid potato across environments. Crop Sci. 34:1361-1368.

Gomez, M.I., M.N. Islam-Faridi, S.S. Woo, and K.F. Schertz. 1997. FISH of a maize sh2-slected sorghum BAC to chromosomes of *Sorghum bicolor*. Genome 40:475-478.

Graham, D., and K.J. Lessman. 1966. Effect of height on yield and yield components of two isogenic lines of *Sorghum vulgare*. Crop Sci. 6:372-374.

Hart, G.E., K.F. Schertz, Y. Peng, and N.H. Syed. 2001. Genetic mapping of *Sorghum bicolor* (L.) Moench QTLs that control variation in tillering and other morphological characters. Theor. Appl. Genet. 103:1232-1242.

Holland, J.B., H.S. Moser, L.S. O'Donoughue, and M. Lee. 1997. QTLs and epistasis associated with vernalization responses in oat. Crop Sci. 37:1306-1316.

Holland, J.B. 1998. EPISTACY: A SAS program for detecting two-locus epistatic interactions using genetic marker information. J. Hered. 89:374-375.

Holland, J.B. 2002. Estimating genotypic and phenotypic correlations and their standard errors using multivariate restricted maximum likelihood estimation in SAS Proc MIXED. Crop Sci. (Submitted).

Holland, J.B., W.E. Nyquist, and C.T. Cervantes-Martinez. 2003. Estimating and interpreting heritability for plant breeding: An update. Plant Breed. Rev. 22:9-111.

House, L.R. 1985. A guide to sorghum breeding. 2nd ed. ICRISAT. Patancheru, India.

Hulbert, S.H., T.E. Richter, J.D. Axtell, and J.L. Bennetzen. 1990. Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. Proc. Natl. Acad. Sci. USA 87:4251-4255.

Johnson, H.W., H.F. Robinson, and R.E. Comstock. 1955. Estimates of genetic and environmental variability in soybeans. Agron. J. 47:314-318.

Johnson, J.W., D.T. Rosenow, G.L. Teetes, and J.M. Phillips. 1982. Registration of 19 greenbug resistant sorghum lines. Crop Sci. 22:1272.

Kebede, H., P.K. Subudhi, D.T. Rosenow, and H.T. Nguyen. 2001. Quantitative trait loci influencing drought tolerance in grain sorghum (*Sorghum bicolor* L. Moench). Theor. Appl. Genet. 103:266-276.

Kimber, C.T. 2000. Origins of domesticated sorghum and its early diffusion to India and China. p. 3-98. *In* Smith et al. (ed.) Sorghum: Origin, history, technology, and production. John Wiley & Sons, Inc., New York.

King, J.G., J.R. Quinby, J.C. Stephens, N.W. Kramer, and K.A. Lahr. 1961. An evaluation of parents of grain sorghum hybrids. The Agricultural and Mechanical College of Texas, Texas Agricultural Experiment Station, in cooperation with the U.S. Department of Agriculture.

Klein, P.E., R.R. Klein, S.W. Cartinhour, P.E. Ulanch, J. Dong, J. Obert, D.R. Morshige, S.D. Schlueter, K.L. Childs, M. Ale, and J.E. Mullet. 2000. A high-throughput AFLP-based method for constructing integrated genetic physical maps: Progress towards a sorghum genome map. Genome Res. 10:789-807.

Klein, R.R., R. Rodriguez-Herrera, J.A. Schulueter, P.E. Klein, Z.H. Yu, and W.L. Rooney. 2001. Identification of genomic regions that affect grain-mould incidence and other traits of agronomic performance in sorghum. Theor. Appl. Genet. 102:307-319.

Knapp, S.J., W.W. Stroup, and W.M. Ross. 1985. Exact confidence intervals for heritability on a progeny mean basis. Crop Sci. 25:192-194.

Lander, E.S., and D. Botstein. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.

Li, Z.K., S.R.M. Pinson, W.D. Park, A.H. Paterson, and J.W. Stansel. 1997. Epistasis for three grain yield components in rice *Oryza sativa* L. Genetics 145:453-465.

Liang, G.H.L., T.L. Walter, C.D. Nickell, and Y.O. Koh. 1969. Heritability estimates and interrelationships among agronomic traits in grain sorghum, *Sorghum bicolor* (L.) Moench. Can. J. Genet. Cytol. 11:199-208.

Liao, C.Y., P. Wu, B. Hu, and K.K. Yi. 2001. Effects of genetic background and environment on QTLs and epistasis for rice (*Oriza sativa* L.) panicle number. Theor. Appl. Genet. 103:104-111.

Lin, Y-R., K.F. Schertz, and A.H. Paterson. 1995. Comparative analysis of QTLs affecting plant height and maturity across the Poaceae, in reference to an interspecific sorghum population. Genetics 141:391-411.

Lothrop, J.E., R.E. Atkins, and O.S. Smith. 1985a. Variability for yield and yield components in IAP1R grain sorghum random-mating population. I. Means, variance components, and heritabilities. Crop Sci. 25:235-240.

Lothrop, J.E., R.E. Atkins, and O.S. Smith. 1985b. Variability for yield and yield components in IAP1R grain sorghum random-mating population. II. Correlations, estimated gains from selection, and correlated responses to selection. Crop Sci. 25: 240-244.

Martin, J.H. 1936. Sorghum improvement. USDA yearbook of agri. 1936.

Maunder, A.B. 1999. History of cultivar development in the United States: From "memoirs of A.B. Maunder-sorghum breeder". p. 191-223. *In* Smith et al. (ed.) Sorghum: Origin, history, technology, and production. John Wiley & Sons, Inc., New York.

Meadow, R.H. 1996. The origin and spread of agriculture and pastoralism in north-western South Asia. p. 390-412. *In* D.R. Harris (ed.) The origins and spread of agriculture and pastoralism in Eurasia. UCL Press, London.

Menz, M.A., R.R. Klein, J.E. Mullet, J.A. Obert, N.C. Unruh, and P.E. Klein. 2002. A high-density genetic map of *Sorghum bicolor* (L.) Moench based on 2926 AFLP®, RFLP and SSR markers. Plant Mol. Biol. 48:483-499.

Menz, M.A., R.R. Klein, P.E. Klein, N.A. Unruh, W.L. Rooney, and J.E. Mullet. 2003. Genetic diversity of public inbreds of sorghum and chromosome haplotyping of lines BTx623 and RTx430. Crop Sci. (Submitted).

Miller, F.R. 1984. Registration of RTx430 sorghum parental line. Crop Sci. 24:1224.

Nyquist, W.E. 1991. Estimation of heritability and prediction of selection response in plant populations. Crit. Rev. Plant Sci. 10:235-322.

Paterson, A.H., S. Damon, J.D. Hewitt, D. Zamir, H.D. Rabinowitch, S.E. Lincoln, E.S Lander, and S.D. Tanksley. 1991a. Mendelian factors underlying quantitative traits in tomato: Comparison across species, generations, and environments. Genetics 127:181-197.

Paterson, A.H., E.S. Lander, J.D. Hewitt, S. Peterson, S.E. Lincoln, and S.D. Tanksley. 1988. Resolution of quantitative traits into mendelian factors using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721-736.

Paterson, A.H., K.F. Schertz, Y-R. Lin, S-C. Liu, and Y-L. Chang. 1995. The weediness of wild plants: Molecular analysis of genes influencing dispersal and persistence of johnsongrass, *Sorghum halapense* (L.) Pers. Proc. Natl. Acad. Sci. USA. 92:6127-6131.

Paterson, A.H.; K.F. Schertz, Y-R. Lin, and Z. Li. 1998. Case history in plant domestication: Sorghum, an example of cereal evolution. p. 187-195. *In* A.H. Paterson (ed.) Molecular dissection of complex traits. CRC Press, Boca Raton, FL.

Paterson, A.H., S.D., Tanksley, and M.E. Sorrells. 1991b. DNA markers in plant improvement. Adv. Agron. 46:39-90.

Peng, Y., K.F. Schertz, S. Cartinhour, and G.E. Hart. 1999. Comparative genome mapping of *Sorghum bicolor* (L.) Moench using an RFLP map constructed in a population of recombinant inbred lines. Plant Breed. 118:225-235.

Pereira, M.G., and M. Lee. 1995. Identification of genomic regions affecting plant height in sorghum and maize. Theor. Appl. Genet. 90:380-388.

Pereira, M.G., M. Lee, P. Bramel-Cox, W. Woodman, J. Doebley, and R. Whitkus. 1994. Construction of an RFLP map using sorghum and maize DNA probes. Genome 37:590-594.

Qiao, K., and W. Zhenshan (ed.) 1970. Varieties of sorghum in China. Agricultural Publishing, Beijing.

Quinby, J.R. 1975. The genetics of sorghum improvement. J. Hered. 66:56-62.

Quinby, J.R., and R.E. Karper. 1945. The inheritance of three genes that influence time of floral initiation and maturity date in milo. J. Am. Soc. Agron. 37:916-936.

Ragab, R.A., S. Dronavalli, M.A. Saghai Maroof, and Y.G. Yu. 1994. Construction of sorghum RFLP linkage map using sorghum and maize DNA probes. Genome. 37:590-594.

Rami, J-F., P. Dufour, G. Trouche, G. Fliedel, C. Mestres, F. Davrieux, P. Blanchard, and P. Hamon. 1998. Quantitative trait loci for grain quality, productivity, morphological and agronomical traits in sorghum (*Sorghum bicolor* L. Moench). Theor. Appl. Genet. 97:605-616.

Rasmusson, D.C., and R.L. Phillips. 1997. Plant breeding progress and genetic diversity from de novo variation and elevated epistasis. Crop Sci. 37:303-310.

Rosenow, D.T., G. Ejeta, L.E. Clark, M.L. Gilbert, R.G. Henzell, A.K. Borell, and R.C. Muchow. 1996. Breeding for pre- and post-flowering drought stress resistance in sorghum. p. 400-411. *In* Rosenow et al. (ed) Proc. Int. Conf. on Genetic Improvement of Sorghum and Pearl Millet. Lubbock, TX. September 23-27. 1996. INTSORMIL.

Rosenow, D.T., J.E. Quiseberry, C.W. Wendt, and L.E. Clark. 1983. Drought tolerant sorghum and cotton germplasm. Agr. Water Mgmt. 7:207-222.

Sanchez-Gomez, A. 2002. Identification of quantitative trait loci for grain yield in a recombinant inbred B-line population in sorghum. Ph.D. Dissertation. Texas A&M University, College Station.

Steel, R.G.D., and J.H. Torrie. 1980. Principle and procedures of statistics. 2nd ed. McGraw-Hill, New York.

Stephens, J.C., and R.F. Holland. 1954. Cytoplasmic male-sterility for hybrid sorghum seed production. Agron. J. 46:20-23.

Stuber, C.W. 1992. Biochemical and molecular markers in plant breeding. Plant Breed. Rev. 9:37-61.

Stuber, C.W. 1999. Heterosis in plant breeding. Plant Breed. Rev. 12:227-251.

Stuber, C.W., S.E. Lincoln, D.W. Wolff, T. Helentjaris, and E.S. Lander. 1992. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:823-839.

Subudhi, P.K., and H.T. Nguyen. 2000. New horizons in biotechnology. p. 349-397. *In* Smith et al. (ed.) Sorghum: Origin, history, technology, and production. John Wiley & Sons, Inc., New York.

Swanson, A.F., and H.H. Laude. 1951. Grain and forage sorghums for Kansas. Kansas St. Univ. Agri. Expt. Sta. Bul. 349.

Tanksley, S.D., N.D. Young, A.H. Paterson, and M.W. Bonierbale. 1989. RFLP mapping in plant breeding: New tool for an old science. Biotechnology 7:257-264.

Tao Y., J.M. Manners, M.M. Ludlow, and R.G. Henzell. 1993. DNA polymorphisms in grain sorghum (*Sorghum bicolor* (L.) Moench). Theor. Appl. Genet. 86:679-688.

Torres-Montalvo, H., L. Mendoza-Onofre, V. Gonzalez-Hernandez, and H. Williams-Alanis. 1992. Reaction of tan and non-tan isogenic genotypes to head blight. Sorghum Newslett. 33:36.

Troyer, A.F. 1999. Background of U.S. hybrid corn. Crop Sci. 39:601-626.

Tuinstra, M.R., E.M. Grote, P.B. Goldsbrough, and G. Ejeta. 1996. Identification of quantitative trait loci associated with preflowering drought tolerance in sorghum. Crop Sci. 36:1337-1344.

Tuinstra, M.R., E.M. Grote, P.B. Goldsbrough, and G. Ejeta. 1997. Genetic analysis of post-flowering drought tolerance and components of grain development in *Sorghum bicolor* (L.) Moench. Mol. Breed. 439:448.

Unger, P.W., and R.L. Baumhardt. 1999. Factors related to dryland grain sorghum yield increases: 1939 through 1997. Agron. J. 91:870-875.

Velboom, L.R., and M. Lee. 1996. Genetic mapping of quantitative trait loci in maize in stress and nonstress environments: I. Grain yield and yield components. Crop Sci. 36:1310-1319.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. Nucl. Acids Res. 23:4407-4414.

Whitkus, R., J. Doebley, and M. Lee. 1992. Comparative genome mapping of sorghum and maize. Genetics 132:1119-1130.

Wooten, D.R. 2001. The use of *Sorghum propinquum* to enhance agronomic traits in sorghum. M.S. Thesis. Texas A&M University, College Station.

Worland, A.J., and C.N. Law. 1986. Genetic analysis of chromosome *2D* of wheat. I. The location of genes affecting height, day-length insensitivity, hybrid dwarfism and yellow-rust resistance. Z. Pflanzenzuecht. 96:331-345.

Xing, Y.Z., Y.F. Tan, J.P. Hua, X.L. Sun, C.G. Xu, and Q. Zhang. 2002. Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. Theor. Appl. Genet. 105:248-257.

Xu, G.W., C.W. Magill, K.F. Schertz, and G.E. Hart. 1994. A RFLP linkage map of *Sorghum bicolor* (L.) Moench. Theor. Appl. Genet. 89:139-145.

Xu, Y. 2002. Global view of QTL: Rice as a model. p. 109-134. *In* M.S. Kang (ed.) Quantitative genetics, genomics and plant breeding. CAB International, Cambridge.

Zeng, Z-B. 1994. Precision mapping of quantitative trait loci. Genetics 136: 1457-1468.

Zher, B.E., J.W. Dudley, J. Chojecki, M.A. Saghai Maroof, and R.P. Mowers. 1992. Use of RFLP markers to search for alleles in a maize population for improvement of an elite hybrid. Theor. Appl. Genet. 83:903-911.

Zhuang, J-Y., H-X. Lin, J. Lu, H-R. Qian, S. Hittalmani, N. Huang, and K-L. Zheng. 1997. Analysis of QTL x environment interaction for yield components and plant height in rice. Theor. Appl. Genet. 95:799-808.

APPENDIX

A1. PEDIGREE OF 187 RECOMBINANT INBRED LINES UTILIZED TO IDENTIFY QUANTITATIVE TRAIT LOCI FOR SEVEN AGRONOMIC TRAITS IN SORGHUM

RLINE	PEDIGREE
R1	(RTX430*TX7000)-CF2-C135-C1-CS1
R2	(RTX7000*TX430)-CF2-C13-C1-CS1
R3	(RTX7000*TX430)-CF2-C49-C1-CS1
R4	(RTX7000*TX430)-CF2-C90-C1-CS1
R5	(RTX7000*TX430)-CF2-C132-C1-CS1
R6	(RTX430*TX7000)-CF2-C35-C1-CS1
R7	(RTX430*TX7000)-CF2-C106-C1-CS1
R8	(RTX430*TX7000)-CF2-C136-C1-CS1
R9	(RTX7000*TX430)-CF2-C14-C1-CS1
R10	(RTX7000*TX430)-CF2-C50-C1-CS1
R11	(RTX7000*TX430)-CF2-C93-C1-CS1
R12	(RTX7000*TX430)-CF2-C133-C1-CS1
R13	(RTX430*TX7000)-CF2-C1-C1-CS1
R14	(RTX430*TX7000)-CF2-C36-C1-CS1
R15	(RTX430*TX7000)-CF2-C107-C1-CS1
R16	(RTX430*TX7000)-CF2-C137-C1-CS1
R17	(RTX7000*TX430)-CF2-C17-C1-CS1
R18	(RTX7000*TX430)-CF2-C52-C1-CS1
R19	(RTX7000*TX430)-CF2-C97-C1-CS1
R20	(RTX7000*TX430)-CF2-C134-C1-CS1
R21	(RTX430*TX7000)-CF2-C2-C1-CS1
R22	(RTX430*TX7000)-CF2-C37-C1-CS1
R23	(RTX430*TX7000)-CF2-C108-C1-CS1
R24	(RTX430*TX7000)-CF2-C138-C1-CS1
R25	(RTX7000*TX430)-CF2-C18-C1-CS1
R26	(RTX7000*TX430)-CF2-C98-C1-CS1
R27	(RTX7000*TX430)-CF2-C135-C1-CS1
R28	(RTX430*TX7000)-CF2-C3-C1-CS1
R29	(RTX430*TX7000)-CF2-C38-C1-CS1
R30	(RTX7000*TX430)-CF2-C19-C1-CS1
R31	(RTX7000*TX430)-CF2-C58-C1-CS1
R32	(RTX7000*TX430)-CF2-C100-C1-CS1
R33	(RTX7000*TX430)-CF2-C136-C1-CS1
R35	(RTX430*TX7000)-CF2-C4-C1-CS1
R36	(RTX430*TX7000)-CF2-C40-C1-CS1
R37	(RTX430*TX7000)-CF2-C111-C1-CS1
R38	(RTX430*TX7000)-CF2-C140-C1-CS1
R39	(RTX7000*TX430)-CF2-C20-C1-CS1
R40	(RTX7000*TX430)-CF2-C61-C1-CS1
R41	(RTX7000*TX430)-CF2-C101-C1-CS1
R42	(RTX7000*TX430)-CF2-C138-C1-CS1
R43	(RTX430*TX7000)-CF2-C7-C1-CS1
R44	(RTX430*TX7000)-CF2-C41-C1-CS1
R45	(RTX430*TX7000)-CF2-C112-C1-CS1
R46	(RTX430*TX7000)-CF2-C143-C1-CS1

RLINE	PEDIGREE
R47	(RTX7000*TX430)-CF2-C21-C1-CS1
R48	(RTX7000*TX430)-CF2-C62-C1-CS1
R49	(RTX7000*TX430)-CF2-C102-C1-CS1
R50	(RTX7000*TX430)-CF2-C139-C1-CS1
R51	(RTX430*TX7000)-CF2-C7-C1-CS1
R52	(RTX430*TX7000)-CF2-C46-C1-CS1
R53	(RTX430*TX7000)-CF2-C114-C1-CS1
R54	(RTX430*TX7000)-CF2-C144-C1-CS1
R55	(RTX7000*TX430)-CF2-C24-C1-CS1
R56	(RTX7000*TX430)-CF2-C63-C1-CS1
R57	(RTX7000*TX430)-CF2-C104-C1-CS1
R58	(RTX7000*TX430)-CF2-C140-C1-CS1
R59	(RTX430*TX7000)-CF2-C8-C1-CS1
R60	(RTX430*TX7000)-CF2-C47-C1-CS1
R61	(RTX430*TX7000)-CF2-C115-C1-CS1
R62	(RTX430*TX7000)-CF2-C145-C1-CS1
R63	(RTX7000*TX430)-CF2-C25-C1-CS1
R64	(RTX7000*TX430)-CF2-C64-C1-CS1
R65	(RTX7000*TX430)-CF2-C105-C1-CS1
R66	(RTX7000*TX430)-CF2-C142-C1-CS1
R67	(RTX430*TX7000)-CF2-C9-C1-CS1
R68	(RTX430*TX7000)-CF2-C48-C1-CS1
R69	(RTX430*TX7000)-CF2-C116-C1-CS1
R70	(RTX430*TX7000)-CF2-C146-C1-CS1
R71	(RTX7000*TX430)-CF2-C26-C1-CS1
R72	(RTX7000*TX430)-CF2-C65-C1-CS1
R73	(RTX7000*TX430)-CF2-C143-C1-CS1
R74	(RTX430*TX7000)-CF2-C11-C1-CS1
R75	(RTX430*TX7000)-CF2-C49-C1-CS1
R76	(RTX430*TX7000)-CF2-C117-C1-CS1
R77	(RTX430*TX7000)-CF2-C147-C1-CS1
R78	(RTX7000*TX430)-CF2-C28-C1-CS1
R79	(RTX7000*TX430)-CF2-C67-C1-CS1
R80	(RTX7000*TX430)-CF2-C108-C1-CS1
R81	(RTX7000*TX430)-CF2-C145-C1-CS1
R82	(RTX430*TX7000)-CF2-C12-C1-CS1
R83	(RTX430*TX7000)-CF2-C50-C1-CS1
R84	(RTX430*TX7000)-CF2-C119-C1-CS1
R85	(RTX430*TX7000)-CF2-C148-C1-CS1
R86	(RTX7000*TX430)-CF2-C68-C1-CS1
R87	(RTX7000*TX430)-CF2-C109-C1-CS1
R88	(RTX7000*TX430)-CF2-C146-C1-CS1
R89	(RTX430*TX7000)-CF2-C15-C1-CS1
R90	(RTX430*TX7000)-CF2-C51-C1-CS1
R91	(RTX430*TX7000)-CF2-C120-C1-CS1

RLINE	PEDIGREE
R92	(RTX430*TX7000)-CF2-C149-C1-CS1
R93	(RTX7000*TX430)-CF2-C31-C1-CS1
R94	(RTX7000*TX430)-CF2-C70-C1-CS1
R95	(RTX7000*TX430)-CF2-C111-C1-CS1
R96	(RTX7000*TX430)-CF2-C147-C1-CS1
R97	(RTX430*TX7000)-CF2-C121-C1-CS1
R98	(RTX430*TX7000)-CF2-C150-C1-CS1
R99	(RTX7000*TX430)-CF2-C33-C1-CS1
R100	(RTX7000*TX430)-CF2-C71-C1-CS1
R101	(RTX7000*TX430)-CF2-C113-C1-CS1
R102	(RTX7000*TX430)-CF2-C149-C1-CS1
R103	(RTX430*TX7000)-CF2-C17-C1-CS1
R104	(RTX430*TX7000)-CF2-C56-C1-CS1
R105	(RTX430*TX7000)-CF2-C151-C1-CS1
R106	(RTX7000*TX430)-CF2-C34-C1-CS1
R107	(RTX7000*TX430)-CF2-C72-C1-CS1
R108	(RTX7000*TX430)-CF2-C115-C1-CS1
R109	(RTX7000*TX430)-CF2-C151-C1-CS1
R110	(RTX430*TX7000)-CF2-C21-C1-CS1
R111	(RTX430*TX7000)-CF2-C57-C1-CS1
R112	(RTX430*TX7000)-CF2-C123-C1-CS1
R113	(RTX430*TX7000)-CF2-C152-C1-CS1
R114	(RTX7000*TX430)-CF2-C35-C1-CS1
R115	(RTX7000*TX430)-CF2-C73-C1-CS1
R116	(RTX7000*TX430)-CF2-C116-C1-CS1
R117	(RTX7000*TX430)-CF2-C153-C1-CS1
R118	(RTX430*TX7000)-CF2-C23-C1-CS1
R119	(RTX430*TX7000)-CF2-C58-C1-CS1
R120	(RTX430*TX7000)-CF2-C124-C1-CS1
R121	(RTX7000*TX430)-CF2-C1-C1-CS1
R122	(RTX7000*TX430)-CF2-C36-C1-CS1
R123	(RTX7000*TX430)-CF2-C75-C1-CS1
R124	(RTX7000*TX430)-CF2-C117-C1-CS1
R125	(RTX7000*TX430)-CF2-C154-C1-CS1
R126	(RTX430*TX7000)-CF2-C24-C1-CS1
R127	(RTX430*TX7000)-CF2-C59-C1-CS1
R128	(RTX430*TX7000)-CF2-C125-C1-CS1
R129	(RTX7000*TX430)-CF2-C2-C1-CS1
R130	(RTX7000*TX430)-CF2-C38-C1-CS1
R131	(RTX7000*TX430)-CF2-C76-C1-CS1
R132	(RTX7000*TX430)-CF2-C118-C1-CS1
R133	(RTX7000*TX430)-CF2-C155-C1-CS1
R134	(RTX430*TX7000)-CF2-C25-C1-CS1
R135	(RTX430*TX7000)-CF2-C60-C1-CS1
R136	(RTX430*TX7000)-CF2-C127-C1-CS1

RLINE	PEDIGREE
R137	(RTX7000*TX430)-CF2-C3-C1-CS1
R138	(RTX7000*TX430)-CF2-C39-C1-CS1
R139	(RTX7000*TX430)-CF2-C77-C2-CS1
R140	(RTX7000*TX430)-CF2-C119-C1-CS1
R141	(RTX7000*TX430)-CF2-C157-C1-CS1
R142	(RTX430*TX7000)-CF2-C26-C1-CS1
R143	(RTX430*TX7000)-CF2-C61-C1-CS1
R144	(RTX430*TX7000)-CF2-C128-C1-CS1
R145	(RTX7000*TX430)-CF2-C5-C1-CS1
R146	(RTX7000*TX430)-CF2-C41-C1-CS1
R147	(RTX7000*TX430)-CF2-C78-C1-CS1
R148	(RTX7000*TX430)-CF2-C120-C1-CS1
R149	(RTX7000*TX430)-CF2-C158-C1-CS1
R150	(RTX430*TX7000)-CF2-C27-C1-CS1
R151	(RTX430*TX7000)-CF2-C97-C1-CS1
R152	(RTX430*TX7000)-CF2-C129-C1-CS1
R153	(RTX7000*TX430)-CF2-C6-C1-CS1
R154	(RTX7000*TX430)-CF2-C43-C1-CS1
R155	(RTX7000*TX430)-CF2-C79-C1-CS1
R156	(RTX7000*TX430)-CF2-C122-C1-CS1
R157	(RTX7000*TX430)-CF2-C159-C1-CS1
R158	(RTX430*TX7000)-CF2-C28-C1-CS1
R159	(RTX430*TX7000)-CF2-C98-C1-CS1
R160	(RTX430*TX7000)-CF2-C131-C1-CS1
R161	(RTX7000*TX430)-CF2-C7-C1-CS1
R162	(RTX7000*TX430)-CF2-C44-C1-CS1
R163	(RTX7000*TX430)-CF2-C81-C1-CS1
R164	(RTX7000*TX430)-CF2-C124-C1-CS1
R165	(RTX7000*TX430)-CF2-C161-C1-CS1
R166	(RTX430*TX7000)-CF2-C29-C1-CS1
R167	(RTX430*TX7000)-CF2-C101-C1-CS1
R168	(RTX430*TX7000)-CF2-C132-C1-CS1
R169	(RTX7000*TX430)-CF2-C8-C1-CS1
R170	(RTX7000*TX430)-CF2-C45-C1-CS1
R171	(RTX7000*TX430)-CF2-C83-C1-CS1
R172	(RTX7000*TX430)-CF2-C126-C1-CS1
R173	(RTX7000*TX430)-CF2-C162-C1-CS1
R174	(RTX430*TX7000)-CF2-C31-C1-CS1
R175	(RTX430*TX7000)-CF2-C102-C1-CS1
R176	(RTX7000*TX430)-CF2-C10-C1-CS1
R177	(RTX7000*TX430)-CF2-C47-C1-CS1
R178	(RTX7000*TX430)-CF2-C88-C1-CS1
R179	(RTX7000*TX430)-CF2-C127-C1-CS1
R180	(RTX7000*TX430)-CF2-C163-C1-CS1
R181	(RTX430*TX7000)-CF2-C32-C1-CS1

RLINE	PEDIGREE
R182	(RTX430*TX7000)-CF2-C103-C1-CS1
R183	(RTX430*TX7000)-CF2-C134-C1-CS1
R184	(RTX7000*TX430)-CF2-C12-C1-CS1
R185	(RTX7000*TX430)-CF2-C48-C1-CS1

RLINE	PEDIGREE
R186	(RTX7000*TX430)-CF2-C130-C1-CS1
R187	(RTX7000*TX430)-CF2-C165-C1-CS1
R188	R.Tx430
R189	R.Tx7000

A2. STATISTICAL OUPUTS OF QTL AND EPISTATIC LINEAR ADDITIVE MODELS FOR SEVEN PHENOTYPIC TRAITS

Dependent Variable: GYL CSI99

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	6.5079160	6.5079160	6.02	0.0153
Error	147	159.0266249	1.0818138		
Corrected Total	148	165.5345409			

R-Square	Coeff Var	Root MSE	YLDCI9 Mean
0.039315	31.58507	1.040103	3.293020

Source	DF	Type III SS	Mean Square	F Value	Pr > F
O239	1	6.50791600	6.50791600	6.02	0.0153

Dependent Variable: GYL BEI99

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	3.40034150	3.40034150	11.70	0.0008
Error	141	40.97867388	0.29062889		
Corrected Total	142	44.37901538			

R-Square	Coeff Var	Root MSE	YLDBI9 Mean
0.076620	35.68381	0.539100	1.510769

Source	DF	Type III SS	Mean Square	F Value	Pr > F
207	1	3.40034150	3.40034150	11.70	0.0008

Dependent Variable: GYL CSI00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	32.1764544	16.0882272	11.65	<.0001
Error	137	189.1450342	1.3806207		
Corrected Total	139	221.3214886			

R-Square	Coeff Var	Root MSE	YLDCI0 Mean
0.145383	33.63590	1.174998	3.493286

Source	DF	Type III SS	Mean Square	F Value	Pr > F
214	1	26.19755860	26.19755860	18.98	<.0001
148	1	11.57473914	11.57473914	8.38	0.0044

Dependent Variable: GYL RIL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	9.76809298	4.88404649	10.24	<.0001
Error	133	63.42853055	0.47690624		
Corrected Total	135	73.19662353			

R-Square	Coeff Var	Root MSE	YLDINB Mean
0.133450	24.91231	0.690584	2.772059

Source	DF	Type III SS	Mean Square	F Value	Pr > F
231	1	6.59742296	6.59742296	13.83	0.0003
270	1	2.98995547	2.98995547	6.27	0.0135

Dependent Variable: GYL RIL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	20	37.10110515	1.85505526	8.20	<.0001
Error	88	19.91182696	0.22627076		
Corrected Total	108	57.01293211			

R-Square	Coeff Var	Root MSE	YLDINB Mean
0.650749	17.59384	0.475679	2.703670

Source	DF	Type III SS	Mean Square	F Value	Pr > F
231	1	2.69874230	2.69874230	11.93	0.0009
270	1	1.64003386	1.64003386	7.25	0.0085
124	1	0.02816585	0.02816585	0.12	0.7251
56	1	0.79176375	0.79176375	3.50	0.0647
124*56	1	4.52684895	4.52684895	20.01	<.0001
129	1	0.61643723	0.61643723	2.72	0.1024
204	1	0.13784998	0.13784998	0.61	0.4372
129*204	1	2.02285074	2.02285074	8.94	0.0036
139	1	0.00272032	0.00272032	0.01	0.9129
156	1	0.00000468	0.00000468	0.00	0.9964
139*156	1	1.99230740	1.99230740	8.80	0.0039
225	1	0.05138266	0.05138266	0.23	0.6349
255	1	0.01171038	0.01171038	0.05	0.8206
225*255	1	0.89550680	0.89550680	3.96	0.0498
145	1	1.02287165	1.02287165	4.52	0.0363
193	1	0.83179162	0.83179162	3.68	0.0584
145*193	1	1.31184244	1.31184244	5.80	0.0181
11	1	0.32804842	0.32804842	1.45	0.2318
84	1	0.03054314	0.03054314	0.13	0.7142
11*84	1	1.28227706	1.28227706	5.67	0.0194

Dependent Variable: GYL CSH00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.90376447	2.90376447	4.54	0.0347
Error	145	92.67186138	0.63911629		
Corrected Total	146	95.57562585			

R-Square	Coeff Var	Root MSE	YLDCH0 Mean
0.030382	14.65376	0.799447	5.455578

Source	DF	Type III SS	Mean Square	F Value	Pr > F
99	1	2.90376447	2.90376447	4.54	0.0347

Dependent Variable: GYL TAH00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	9.9674261	4.9837131	7.56	0.0008
Error	143	94.2447958	0.6590545		
Corrected Total	145	104.2122219			

R-Square	Coeff Var	Root MSE	YLDTH0 Mean
0.095645	14.67160	0.811822	5.533288

Source	DF	Type III SS	Mean Square	F Value	Pr > F
135	1	4.54373024	4.54373024	6.89	0.0096
348	1	6.63616807	6.63616807	10.07	0.0018

Dependent Variable: GYL CSH01

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	5.59428790	5.59428790	10.67	0.0014
Error	144	75.46951005	0.52409382		
Corrected Total	145	81.06379795			

R-Square	Coeff Var	Root MSE	YLDCH1 Mean
0.069011	16.83026	0.723943	4.301438

Source	DF	Type III SS	Mean Square	F Value	Pr > F
264	1	5.59428790	5.59428790	10.67	0.0014

Dependent Variable: GYL TCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	6.90141326	2.30047109	9.22	<.0001
Error	137	34.18832717	0.24954983		
Corrected Total	140	41.08974043			

R-Square	Coeff Var	Root MSE	YLDHYB Mean
0.167960	9.777958	0.499550	5.108936

Source	DF	Type III SS	Mean Square	F Value	Pr > F
344	1	2.08331479	2.08331479	8.35	0.0045
284	1	1.69890414	1.69890414	6.81	0.0101
207	1	2.44689739	2.44689739	9.81	0.0021

Dependent Variable: GYL TCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	14	16.93476619	1.20962616	7.44	<.0001
Error	108	17.56803544	0.16266699		
Corrected Total	122	34.50280163			

R-Square	Coeff Var	Root MSE	YLDHYB Mean
0.490823	7.875967	0.403320	5.120894

Source	DF	Type III SS	Mean Square	F Value	Pr > F
344	1	1.33680732	1.33680732	8.22	0.0050
284	1	2.24677779	2.24677779	13.81	0.0003
207	1	1.01303507	1.01303507	6.23	0.0141
225	1	0.10810367	0.10810367	0.66	0.4167
344*225	1	1.26740342	1.26740342	7.79	0.0062
223	1	0.00315826	0.00315826	0.02	0.8894
348	1	0.00013288	0.00013288	0.00	0.9773
223*348	1	2.57839416	2.57839416	15.85	0.0001
146	1	0.14693155	0.14693155	0.90	0.3440
2	1	0.32775896	0.32775896	2.01	0.1586
146*2	1	0.98876370	0.98876370	6.08	0.0153
138	1	2.07126440	2.07126440	12.73	0.0005
145	1	0.18377406	0.18377406	1.13	0.2902
138*145	1	1.44678777	1.44678777	8.89	0.0035

Dependent Variable: PHE CSI99

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	6507.15660	2169.05220	9.47	<.0001
Error	135	30918.22914	229.02392		
Corrected Total	138	37425.38574			

R-Square	Coeff Var	Root MSE	PHECI9 Mean
0.173870	12.06373	15.13354	125.4465

Source	DF	Type III SS	Mean Square	F Value	Pr > F
176	1	2388.146166	2388.146166	10.43	0.0016
215	1	3178.306631	3178.306631	13.88	0.0003
257	1	1004.626004	1004.626004	4.39	0.0381

Dependent Variable: PHE BEI99

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1131.67831	565.83916	5.31	0.0060
Error	139	14799.04104	106.46792		
Corrected Total	141	15930.71936			

R-Square	Coeff Var	Root MSE	PHEBI9 Mean
0.071037	10.67896	10.31833	96.62296

Source	DF	Type III SS	Mean Square	F Value	Pr > F
225	1	567.5750380	567.5750380	5.33	0.0224
4	1	718.9290600	718.9290600	6.75	0.0104

Dependent Variable: PHE CSI00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	6560.03677	2186.67892	11.54	<.0001
Error	136	25759.91008	189.41110		
Corrected Total	139	32319.94685			

R-Square	Coeff Var	Root MSE	PHECI0 Mean
0.202972	11.74875	13.76267	117.1416

Source	DF	Type III SS	Mean Square	F Value	Pr > F
200	1	2177.796760	2177.796760	11.50	0.0009
109	1	2708.911404	2708.911404	14.30	0.0002
18	1	2102.162662	2102.162662	11.10	0.0011

Dependent Variable: PHE RIL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2627.69979	1313.84989	9.85	<.0001
Error	140	18670.53415	133.36096		
Corrected Total	142	21298.23394			

R-Square	Coeff Var	Root MSE	PHEINB Mean
0.123376	10.24281	11.54820	112.7445

Source	DF	Type III SS	Mean Square	F Value	Pr > F
312	1	1675.584470	1675.584470	12.56	0.0005
253	1	851.395636	851.395636	6.38	0.0126

Dependent Variable: PHE RIL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	9234.33702	710.33362	7.78	<.0001
Error	115	10506.09835	91.35738		
Corrected Total	128	19740.43537			

R-Square	Coeff Var	Root MSE	PHEINB Mean
0.467788	8.473422	9.558105	112.8010

Source	DF	Type III SS	Mean Square	F Value	Pr > F
312	1	705.072463	705.072463	7.72	0.0064
253	1	456.252604	456.252604	4.99	0.0274
264	1	0.117790	0.117790	0.00	0.9714
91	1	442.499074	442.499074	4.84	0.0297
264*91	1	753.965113	753.965113	8.25	0.0048
111	1	2.012541	2.012541	0.02	0.8823
235	1	347.883484	347.883484	3.81	0.0534
111*235	1	1161.774056	1161.774056	12.72	0.0005
267	1	197.336695	197.336695	2.16	0.1444
321	1	446.875885	446.875885	4.89	0.0290
267*321	1	1536.364972	1536.364972	16.82	<.0001
97	1	601.164704	601.164704	6.58	0.0116
267*97	1	533.430371	533.430371	5.84	0.0172

Dependent Variable: PHE CSH00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3147.19051	786.79763	8.77	<.0001
Error	130	11662.01317	89.70779		
Corrected Total	134	14809.20368			

R-Square	Coeff Var	Root MSE	PHECH0 Mean
0.212516	7.660968	9.471420	123.6321

Source	DF	Type III SS	Mean Square	F Value	Pr > F
46	1	630.958797	630.958797	7.03	0.0090
284	1	1390.518831	1390.518831	15.50	0.0001
215	1	729.700195	729.700195	8.13	0.0051
126	1	332.379017	332.379017	3.71	0.0564

Dependent Variable: PHE TAH00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	247.46667	247.46667	3.46	0.0649
Error	143	10228.66092	71.52910		
Corrected Total	144	10476.12759			

R-Square	Coeff Var	Root MSE	PHETH0 Mean
0.023622	7.358529	8.457488	114.9345

Source	DF	Type III SS	Mean Square	F Value	Pr > F
329	1	247.4666667	247.4666667	3.46	0.0649

Dependent Variable: PHE CSH01

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3498.32570	874.58143	15.20	<.0001
Error	140	8053.68243	57.52630		
Corrected Total	144	11552.00814			

R-Square	Coeff Var	Root MSE	PHECH1 Mean
0.302833	5.701274	7.584610	133.0336

Source	DF	Type III SS	Mean Square	F Value	Pr > F
22	1	1174.353680	1174.353680	20.41	<.0001
131	1	713.074332	713.074332	12.40	0.0006
284	1	1740.385183	1740.385183	30.25	<.0001
285	1	560.935519	560.935519	9.75	0.0022

Dependent Variable: PHE TCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1683.928660	561.309553	13.54	<.0001
Error	136	5638.716662	41.461152		
Corrected Total	139	7322.645322			

R-Square	Coeff Var	Root MSE	PHEHYB Mean
0.229962	5.199409	6.439033	123.8416

Source	DF	Type III SS	Mean Square	F Value	Pr > F
284	1	839.7452821	839.7452821	20.25	<.0001
70	1	535.5477702	535.5477702	12.92	0.0005
178	1	405.7770433	405.7770433	9.79	0.0022

Dependent Variable: PHE TCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	3241.362490	270.113541	8.78	<.0001
Error	110	3382.594681	30.750861		
Corrected Total	122	6623.957171			

R-Square	Coeff Var	Root MSE	PHEHYB Mean
0.489339	4.475319	5.545346	123.9095

Source	DF	Type III SS	Mean Square	F Value	Pr > F
284	1	355.7993797	355.7993797	11.57	0.0009
70	1	290.9197594	290.9197594	9.46	0.0026
178	1	251.9380787	251.9380787	8.19	0.0050
52	1	290.9755152	290.9755152	9.46	0.0026
97	1	37.9687177	37.9687177	1.23	0.2689
52*97	1	317.8004072	317.8004072	10.33	0.0017
186	1	104.3459537	104.3459537	3.39	0.0682
217	1	430.4646719	430.4646719	14.00	0.0003
186*217	1	237.7636952	237.7636952	7.73	0.0064
111	1	20.1186658	20.1186658	0.65	0.4203
275	1	4.6056429	4.6056429	0.15	0.6995
111*275	1	382.4214770	382.4214770	12.44	0.0006

Dependent Variable: DMA CSI99

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	126.084904	63.042452	6.02	0.0031
Error	143	1496.779000	10.466986		
Corrected Total	145	1622.863904			

R-Square	Coeff Var	Root MSE	DMACI9 Mean
0.077693	4.654884	3.235272	69.50274

Source	DF	Type III SS	Mean Square	F Value	Pr > F
278	1	65.17865580	65.17865580	6.23	0.0137
273	1	76.53089538	76.53089538	7.31	0.0077

Dependent Variable: DMA BEI99

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	415.689091	83.137818	7.45	<.0001
Error	130	1450.587991	11.158369		
Corrected Total	135	1866.277082			

R-Square	Coeff Var	Root MSE	DMABI9 Mean
0.222737	4.666011	3.340415	71.59037

Source	DF	Type III SS	Mean Square	F Value	Pr > F
163	1	168.8683932	168.8683932	15.13	0.0002
261	1	92.4382503	92.4382503	8.28	0.0047
36	1	138.8983865	138.8983865	12.45	0.0006
133	1	63.0963289	63.0963289	5.65	0.0189
273	1	82.9049755	82.9049755	7.43	0.0073

Dependent Variable: DMA CSI00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	104.5964005	34.8654668	7.63	<.0001
Error	141	644.2184243	4.5689250		
Corrected Total	144	748.8148248			

R-Square	Coeff Var	Root MSE	DMACI0 Mean
0.139683	3.707407	2.137504	57.65497

Source	DF	Type III SS	Mean Square	F Value	Pr > F
57	1	48.03587058	48.03587058	10.51	0.0015
333	1	25.48874365	25.48874365	5.58	0.0195
54	1	30.16645009	30.16645009	6.60	0.0112

Dependent Variable: DMA RIL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	299.298384	59.859677	11.45	<.0001
Error	134	700.756538	5.229526		
Corrected Total	139	1000.054922			

R-Square	Coeff Var	Root MSE	DMAINB Mean
0.299282	3.452196	2.286816	66.24236

Source	DF	Type III SS	Mean Square	F Value	Pr > F
163	1	117.2885473	117.2885473	22.43	<.0001
301	1	55.4095520	55.4095520	10.60	0.0014
294	1	42.3278166	42.3278166	8.09	0.0051
278	1	79.7243165	79.7243165	15.25	0.0001
273	1	132.2937584	132.2937584	25.30	<.0001

Dependent Variable: DMA RIL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	518.4019402	30.4942318	8.91	<.0001
Error	108	369.7800256	3.4238891		
Corrected Total	125	888.1819659			

R-Square	Coeff Var	Root MSE	DMAINB Mean
0.583666	2.795930	1.850375	66.18103

Source	DF	Type III SS	Mean Square	F Value	Pr > F
163	1	35.73920890	35.73920890	10.44	0.0016
301	1	18.59824247	18.59824247	5.43	0.0216
294	1	27.65433219	27.65433219	8.08	0.0054
278	1	39.30946468	39.30946468	11.48	0.0010
273	1	18.80927447	18.80927447	5.49	0.0209
165	1	0.37147029	0.37147029	0.11	0.7425
241	1	0.26187407	0.26187407	0.08	0.7826
165*241	1	33.28061355	33.28061355	9.72	0.0023
238	1	15.20964865	15.20964865	4.44	0.0374
41	1	5.99531283	5.99531283	1.75	0.1885
238*41	1	22.77031676	22.77031676	6.65	0.0113
126	1	17.84752256	17.84752256	5.21	0.0244
91	1	23.90993316	23.90993316	6.98	0.0095
126*91	1	32.56909369	32.56909369	9.51	0.0026
1	1	9.82873027	9.82873027	2.87	0.0931
109	1	2.14304532	2.14304532	0.63	0.4306
1*109	1	66.04169905	66.04169905	19.29	<.0001

Dependent Variable: DMA CSH00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	213.1126159	53.2781540	13.50	<.0001
Error	122	481.4834597	3.9465857		
Corrected Total	126	694.5960756			

R-Square	Coeff Var	Root MSE	DMACH0 Mean
0.306815	2.395127	1.986602	82.94346

Source	DF	Type III SS	Mean Square	F Value	Pr > F
309	1	21.4864496	21.4864496	5.44	0.0213
138	1	21.7637991	21.7637991	5.51	0.0205
278	1	40.2712664	40.2712664	10.20	0.0018
245	1	132.4112801	132.4112801	33.55	<.0001

Dependent Variable: DMA TAH00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	72.8490576	36.4245288	16.50	<.0001
Error	138	304.6190275	2.2073843		
Corrected Total	140	377.4680851			

R-Square	Coeff Var	Root MSE	DMATH0 Mean
0.192994	2.171305	1.485727	68.42553

Source	DF	Type III SS	Mean Square	F Value	Pr > F
330	1	61.14580403	61.14580403	27.70	<.0001
245	1	32.55399231	32.55399231	14.75	0.0002

Dependent Variable: DMA CSH01

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	93.3740136	46.6870068	20.76	<.0001
Error	140	314.8941780	2.2492441		
Corrected Total	142	408.2681916			

R-Square	Coeff Var	Root MSE	DMACH1 Mean
0.228708	2.087084	1.499748	71.85853

Source	DF	Type III SS	Mean Square	F Value	Pr > F
329	1	26.30947272	26.30947272	11.70	0.0008
273	1	81.02626434	81.02626434	36.02	<.0001

Dependent Variable: DMA TCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	114.0642831	38.0214277	20.64	<.0001
Error	130	239.4926064	1.8422508		
Corrected Total	133	353.5568896			

R-Square	Coeff Var	Root MSE	DMAHYB Mean
0.322619	1.824673	1.357295	74.38567

Source	DF	Type III SS	Mean Square	F Value	Pr > F
138	1	9.69235183	9.69235183	5.26	0.0234
330	1	38.36064783	38.36064783	20.82	<.0001
273	1	85.58825027	85.58825027	46.46	<.0001

Dependent Variable: DMA TCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	158.4957307	17.6106367	11.66	<.0001
Error	114	172.2074564	1.5105917		
Corrected Total	123	330.7031871			

R-Square	Coeff Var	Root MSE	DMAHYB Mean
0.479269	1.651971	1.229061	74.39968

Source	DF	Type III SS	Mean Square	F Value	Pr > F
138	1	6.82350426	6.82350426	4.52	0.0357
330	1	37.66014313	37.66014313	24.93	<.0001
273	1	56.34887406	56.34887406	37.30	<.0001
11	1	0.59195230	0.59195230	0.39	0.5326
196	1	10.76983152	10.76983152	7.13	0.0087
11*196	1	25.28569172	25.28569172	16.74	<.0001
311	1	7.34120113	7.34120113	4.86	0.0295
316	1	5.09284679	5.09284679	3.37	0.0689
311*316	1	9.88008749	9.88008749	6.54	0.0119

Dependent Variable: PAN CSI99

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	32925541377	16462770689	9.63	0.0001
Error	141	241168643268	1710415909.7		
Corrected Total	143	274094184646			

R-Square	Coeff Var	Root MSE	PANCI9 Mean
0.120125	30.90054	41357.17	133839.6

Source	DF	Type III SS	Mean Square	F Value	Pr > F
46	1	12629194766	12629194766	7.38	0.0074
163	1	18160586564	18160586564	10.62	0.0014

Dependent Variable: PAN BEI99

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	7743107287.8	3871553643.9	5.41	0.0054
Error	144	103124684043	716143639.19		
Corrected Total	146	110867791331			

R-Square	Coeff Var	Root MSE	PANBI9 Mean
0.069841	40.19557	26760.86	66576.65

Source	DF	Type III SS	Mean Square	F Value	Pr > F
22	1	3881283212	3881283212	5.42	0.0213
223	1	4311945001	4311945001	6.02	0.0153

Dependent Variable: PAN CSI00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	5396083419	5396083419	8.61	0.0039
Error	146	91511018601	626787799		
Corrected Total	147	96907102021			

R-Square	Coeff Var	Root MSE	PANCI0 Mean
0.055683	30.33176	25035.73	82539.66

Source	DF	Type III SS	Mean Square	F Value	Pr > F
23	1	5396083419	5396083419	8.61	0.0039

Dependent Variable: PAN RIL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	10220253040	5110126520	8.56	0.0003
Error	144	85976859836	597061527		
Corrected Total	146	96197112876			

R-Square	Coeff Var	Root MSE	PANINB Mean
0.106243	25.85619	24434.84	94502.86

Source	DF	Type III SS	Mean Square	F Value	Pr > F
46	1	6417369876	6417369876	10.75	0.0013
223	1	4391654752	4391654752	7.36	0.0075

Dependent Variable: PAN RIL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	41794298018	2199699896	7.09	<.0001
Error	92	28528845584	310096148		
Corrected Total	111	70323143602			

R-Square	Coeff Var	Root MSE	PANINB Mean
0.594318	18.64000	17609.55	94471.84

Source	DF	Type III SS	Mean Square	F Value	Pr > F
46	1	2004173539	2004173539	6.46	0.0127
223	1	2131899214	2131899214	6.87	0.0102
335	1	1812352149	1812352149	5.84	0.0176
81	1	166426802	166426802	0.54	0.4657
335*81	1	4586370058	4586370058	14.79	0.0002
41	1	128490891	128490891	0.41	0.5214
65	1	259374221	259374221	0.84	0.3628
41*65	1	3805294252	3805294252	12.27	0.0007
195	1	17709100	17709100	0.06	0.8117
36	1	371376092	371376092	1.20	0.2767
195*36	1	4776723940	4776723940	15.40	0.0002
112	1	240625899	240625899	0.78	0.3807
112*195	1	3463847857	3463847857	11.17	0.0012
154	1	451608992	451608992	1.46	0.2306
352	1	156985284	156985284	0.51	0.4786
154*352	1	1854990645	1854990645	5.98	0.0164
126	1	410586685	410586685	1.32	0.2528
216	1	1585859894	1585859894	5.11	0.0261
126*216	1	2230897349	2230897349	7.19	0.0087

Dependent Variable: PAN CSH00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	7233881014	2411293671	7.97	<.0001
Error	132	39922427610	302442633		
Corrected Total	135	47156308624			

R-Square	Coeff Var	Root MSE	PANCH0 Mean
0.153402	16.42777	17390.88	105862.7

Source	DF	Type III SS	Mean Square	F Value	Pr > F
270	1	4518254606	4518254606	14.94	0.0002
261	1	1245178771	1245178771	4.12	0.0445
147	1	1328989332	1328989332	4.39	0.0380

Dependent Variable: PAN CSH01

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	6593483538.7	6593483538.7	6.83	0.0099
Error	145	139908045273	964883070.85		
Corrected Total	146	146501528811			

R-Square	Coeff Var	Root MSE	PANCH1 Mean
0.045006	17.61665	31062.57	176325.0

Source	DF	Type III SS	Mean Square	F Value	Pr > F
147	1	6593483539	6593483539	6.83	0.0099

Dependent Variable: PAN TCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	11456733150	3818911050	12.12	<.0001
Error	142	44740763795	315075801		
Corrected Total	145	56197496945			

R-Square	Coeff Var	Root MSE	PANHYB Mean
0.203866	12.59177	17750.37	140968.0

Source	DF	Type III SS	Mean Square	F Value	Pr > F
104	1	3028653322	3028653322	9.61	0.0023
147	1	3248043555	3248043555	10.31	0.0016
187	1	5751830585	5751830585	18.26	<.0001

Dependent Variable: PAN TCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	24399087169	1626605811	8.42	<.0001
Error	100	19325650476	193256505		
Corrected Total	115	43724737645			

R-Square	Coeff Var	Root MSE	PANHYB Mean
0.558016	9.808124	13901.67	141736.3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
104	1	1211735131	1211735131	6.27	0.0139
147	1	1090560303	1090560303	5.64	0.0194
187	1	2556397253	2556397253	13.23	0.0004
18	1	3029	3029	0.00	0.9968
267	1	1569064825	1569064825	8.12	0.0053
18*267	1	1538088116	1538088116	7.96	0.0058
178	1	364316096	364316096	1.89	0.1728
310	1	17604549	17604549	0.09	0.7634
178*310	1	4703822804	4703822804	24.34	<.0001
148	1	1569653	1569653	0.01	0.9284
214	1	1033427851	1033427851	5.35	0.0228
148*214	1	3549470006	3549470006	18.37	<.0001
278	1	256329203	256329203	1.33	0.2522
86	1	1533235966	1533235966	7.93	0.0058
278*86	1	922738812	922738812	4.77	0.0312

Dependent Variable: PLE CSI99

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	187.2860104	62.4286701	11.62	<.0001
Error	134	719.6092975	5.3702186		
Corrected Total	137	906.8953080			

R-Square	Coeff Var	Root MSE	PLECI9 Mean
0.206513	7.284134	2.317373	31.81399

Source	DF	Type III SS	Mean Square	F Value	Pr > F
46	1	100.4426352	100.4426352	18.70	<.0001
212	1	55.9584282	55.9584282	10.42	0.0016
296	1	25.5334467	25.5334467	4.75	0.0310

Dependent Variable: PLE BEI99

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	157.9834275	52.6611425	8.45	<.0001
Error	132	822.5117225	6.2311494		
Corrected Total	135	980.4951500			

R-Square	Coeff Var	Root MSE	PLEBI9 Mean
0.161126	9.408186	2.496227	26.53250

Source	DF	Type III SS	Mean Square	F Value	Pr > F
46	1	62.45669153	62.45669153	10.02	0.0019
212	1	53.59938781	53.59938781	8.60	0.0040
114	1	25.66783730	25.66783730	4.12	0.0444

Dependent Variable: PLE CSI00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	380.710960	95.177740	15.16	<.0001
Error	131	822.525663	6.278822		
Corrected Total	135	1203.236624			

R-Square	Coeff Var	Root MSE	PLECI0 Mean
0.316406	9.515996	2.505758	26.33206

Source	DF	Type III SS	Mean Square	F Value	Pr > F
105	1	37.8056116	37.8056116	6.02	0.0154
178	1	69.7081779	69.7081779	11.10	0.0011
36	1	112.5783004	112.5783004	17.93	<.0001
333	1	100.8731629	100.8731629	16.07	0.0001

Dependent Variable: PLE RIL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	222.1480047	44.4296009	12.29	<.0001
Error	129	466.2775923	3.6145550		
Corrected Total	134	688.4255970			

R-Square	Coeff Var	Root MSE	PLEINB Mean
0.322690	6.740368	1.901198	28.20615

Source	DF	Type III SS	Mean Square	F Value	Pr > F
46	1	45.08769853	45.08769853	12.47	0.0006
212	1	21.78371920	21.78371920	6.03	0.0154
36	1	40.50494957	40.50494957	11.21	0.0011
114	1	20.50224130	20.50224130	5.67	0.0187
275	1	15.93302772	15.93302772	4.41	0.0377

Dependent Variable: PLE RIL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	14	291.0041089	20.7860078	7.80	<.0001
Error	102	271.8388210	2.6650865		
Corrected Total	116	562.8429299			

R-Square	Coeff Var	Root MSE	PLEINB Mean
0.517025	5.789689	1.632509	28.19684

Source	DF	Type III SS	Mean Square	F Value	Pr > F
46	1	35.67601323	35.67601323	13.39	0.0004
212	1	18.82279583	18.82279583	7.06	0.0091
36	1	19.81162433	19.81162433	7.43	0.0075
114	1	17.44366005	17.44366005	6.55	0.0120
275	1	12.47064543	12.47064543	4.68	0.0329
11	1	8.04898506	8.04898506	3.02	0.0853
215	1	4.17599651	4.17599651	1.57	0.2135
11*215	1	24.97789860	24.97789860	9.37	0.0028
138	1	5.95975898	5.95975898	2.24	0.1379
145	1	18.23593594	18.23593594	6.84	0.0103
138*145	1	15.12272663	15.12272663	5.67	0.0191
277	1	4.95151440	4.95151440	1.86	0.1759
301	1	5.50971691	5.50971691	2.07	0.1535
277*301	1	30.91889668	30.91889668	11.60	0.0009

Dependent Variable: PLE CSH00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	195.2432555	48.8108139	10.09	<.0001
Error	135	653.1450731	4.8381117		
Corrected Total	139	848.3883286			

R-Square	Coeff Var	Root MSE	PLECH0 Mean
0.230134	8.219402	2.199571	26.76071

Source	DF	Type III SS	Mean Square	F Value	Pr > F
157	1	34.32705706	34.32705706	7.10	0.0087
264	1	57.71894425	57.71894425	11.93	0.0007
52	1	46.18989099	46.18989099	9.55	0.0024
333	1	47.33851368	47.33851368	9.78	0.0022

Dependent Variable: PLE CSH01

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	90.1361411	30.0453804	9.31	<.0001
Error	139	448.7053679	3.2280962		
Corrected Total	142	538.8415091			

R-Square	Coeff Var	Root MSE	PLECH1 Mean
0.167278	6.779033	1.796690	26.50364

Source	DF	Type III SS	Mean Square	F Value	Pr > F
36	1	60.91844459	60.91844459	18.87	<.0001
333	1	18.93637056	18.93637056	5.87	0.0167
273	1	22.84920952	22.84920952	7.08	0.0087

Dependent Variable: PLE TCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	53.0817227	26.5408613	9.31	0.0002
Error	140	399.2797976	2.8519986		
Corrected Total	142	452.3615203			

R-Square	Coeff Var	Root MSE	PLEHYB Mean
0.117344	6.336576	1.688786	26.65140

Source	DF	Type III SS	Mean Square	F Value	Pr > F
264	1	21.88672052	21.88672052	7.67	0.0064
333	1	34.16898240	34.16898240	11.98	0.0007

Dependent Variable: PLE TCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	226.0023234	13.2942543	8.08	<.0001
Error	109	179.4086750	1.6459511		
Corrected Total	126	405.4109984			

R-Square	Coeff Var	Root MSE	PLEHYB Mean
0.557465	4.816092	1.282946	26.63874

Source	DF	Type III SS	Mean Square	F Value	Pr > F
264	1	6.58735014	6.58735014	4.00	0.0479
333	1	25.43570767	25.43570767	15.45	0.0001
110	1	0.05418906	0.05418906	0.03	0.8564
303	1	4.09531144	4.09531144	2.49	0.1176
110*303	1	12.51491571	12.51491571	7.60	0.0068
148	1	0.21012529	0.21012529	0.13	0.7216
316	1	0.75226992	0.75226992	0.46	0.5004
148*316	1	9.66107631	9.66107631	5.87	0.0171
34	1	1.87821473	1.87821473	1.14	0.2878
344	1	6.74857829	6.74857829	4.10	0.0453
34*344	1	18.08006572	18.08006572	10.98	0.0012
248	1	19.20103675	19.20103675	11.67	0.0009
80	1	0.37422157	0.37422157	0.23	0.6344
248*80	1	18.67728005	18.67728005	11.35	0.0010
193	1	0.86019638	0.86019638	0.52	0.4713
37	1	16.67243131	16.67243131	10.13	0.0019
193*37	1	9.40666268	9.40666268	5.72	0.0185

Dependent Variable: PEX CSI99

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	740.355589	246.785196	12.74	<.0001
Error	140	2711.423186	19.367308		
Corrected Total	143	3451.778775			

R-Square	Coeff Var	Root MSE	PEXCI9 Mean
0.214485	66.08267	4.400830	6.659583

Source	DF	Type III SS	Mean Square	F Value	Pr > F
146	1	242.7610930	242.7610930	12.53	0.0005
133	1	247.5530251	247.5530251	12.78	0.0005
137	1	293.9832463	293.9832463	15.18	0.0002

Dependent Variable: PEX BEI99

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	894.814727	223.703682	12.74	<.0001
Error	133	2335.319711	17.558795		
Corrected Total	137	3230.134438			

R-Square	Coeff Var	Root MSE	PEXBI9 Mean
0.277021	56.53904	4.190322	7.411377

Source	DF	Type III SS	Mean Square	F Value	Pr > F
59	1	118.5815178	118.5815178	6.75	0.0104
223	1	273.6355519	273.6355519	15.58	0.0001
178	1	135.9520220	135.9520220	7.74	0.0062
133	1	471.3676293	471.3676293	26.85	<.0001

Dependent Variable: PEX CSI00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	289.105424	144.552712	10.23	<.0001
Error	144	2035.496843	14.135395		
Corrected Total	146	2324.602267			

R-Square	Coeff Var	Root MSE	PEXCI0 Mean
0.124368	87.54168	3.759707	4.294762

Source	DF	Type III SS	Mean Square	F Value	Pr > F
130	1	149.9207760	149.9207760	10.61	0.0014
133	1	139.0751678	139.0751678	9.84	0.0021

Dependent Variable: PEX RIL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	388.776053	97.194013	7.88	<.0001
Error	125	1541.204108	12.329633		
Corrected Total	129	1929.980161			

R-Square	Coeff Var	Root MSE	PEXINB Mean
0.201440	56.43106	3.511358	6.222385

Source	DF	Type III SS	Mean Square	F Value	Pr > F
247	1	84.3176068	84.3176068	6.84	0.0100
31	1	80.0925149	80.0925149	6.50	0.0120
178	1	81.4219727	81.4219727	6.60	0.0114
133	1	233.5983627	233.5983627	18.95	<.0001

Dependent Variable: PEX CSH00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	288.525097	144.262549	11.03	<.0001
Error	142	1856.839698	13.076336		
Corrected Total	144	2145.364796			

R-Square	Coeff Var	Root MSE	PEXCH0 Mean
0.134488	55.19576	3.616122	6.551448

Source	DF	Type III SS	Mean Square	F Value	Pr > F
170	1	51.8077363	51.8077363	3.96	0.0485
187	1	237.3526569	237.3526569	18.15	<.0001

Dependent Variable: PEX TAH00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	172.5684468	57.5228156	9.72	<.0001
Error	130	769.2748368	5.9174987		
Corrected Total	133	941.8432836			

R-Square	Coeff Var	Root MSE	PEXTH0 Mean
0.183224	21.86232	2.432591	11.12687

Source	DF	Type III SS	Mean Square	F Value	Pr > F
312	1	53.34445955	53.34445955	9.01	0.0032
217	1	90.35228352	90.35228352	15.27	0.0001
112	1	41.48654344	41.48654344	7.01	0.0091

Dependent Variable: PEX CSH01

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	183.644738	183.644738	12.87	0.0005
Error	145	2069.744390	14.274099		
Corrected Total	146	2253.389128			

R-Square	Coeff Var	Root MSE	PEXCH1 Mean
0.081497	29.32864	3.778108	12.88197

Source	DF	Type III SS	Mean Square	F Value	Pr > F
100	1	183.6447383	183.6447383	12.87	0.0005

Dependent Variable: PEX TCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	219.5875270	54.8968817	11.77	<.0001
Error	131	610.8942841	4.6633151		
Corrected Total	135	830.4818110			

R-Square	Coeff Var	Root MSE	PEXHYB Mean
0.264410	21.09025	2.159471	10.23919

Source	DF	Type III SS	Mean Square	F Value	Pr > F
100	1	70.94585929	70.94585929	15.21	0.0002
200	1	50.60661595	50.60661595	10.85	0.0013
170	1	24.71492299	24.71492299	5.30	0.0229
112	1	58.12665352	58.12665352	12.46	0.0006

Dependent Variable: PEX TCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	260.8604637	37.2657805	8.40	<.0001
Error	121	536.9414122	4.4375323		
Corrected Total	128	797.8018760			

R-Square	Coeff Var	Root MSE	PEXHYB Mean
0.326974	20.42945	2.106545	10.31132

Source	DF	Type III SS	Mean Square	F Value	Pr > F
100	1	74.73436574	74.73436574	16.84	<.0001
200	1	28.51374225	28.51374225	6.43	0.0125
170	1	27.80373267	27.80373267	6.27	0.0136
112	1	30.23061950	30.23061950	6.81	0.0102
247	1	6.91108672	6.91108672	1.56	0.2145
312	1	0.61293109	0.61293109	0.14	0.7108
247*312	1	45.48922151	45.48922151	10.25	0.0017

Dependent Variable: PWE CSI00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3267.88764	816.97191	6.98	<.0001
Error	139	16258.42362	116.96708		
Corrected Total	143	19526.31127			

R-Square	Coeff Var	Root MSE	PWECI0 Mean
0.167358	24.90846	10.81513	43.41951

Source	DF	Type III SS	Mean Square	F Value	Pr > F
321	1	828.2203700	828.2203700	7.08	0.0087
297	1	779.7253532	779.7253532	6.67	0.0109
182	1	832.8427591	832.8427591	7.12	0.0085
181	1	512.3658842	512.3658842	4.38	0.0382

Dependent Variable: PWE RIL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	413.268448	413.268448	9.91	0.0020
Error	145	6046.095013	41.697207		
Corrected Total	146	6459.363461			

R-Square	Coeff Var	Root MSE	PWEINB Mean
0.063980	20.80683	6.457337	31.03469

Source	DF	Type III SS	Mean Square	F Value	Pr > F
133	1	413.2684482	413.2684482	9.91	0.0020

Dependent Variable: PWE RIL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	16	2804.606814	175.287926	6.16	<.0001
Error	94	2675.253685	28.460146		
Corrected Total	110	5479.860499			

R-Square	Coeff Var	Root MSE	PWEINB Mean
0.511803	17.29464	5.334805	30.84658

Source	DF	Type III SS	Mean Square	F Value	Pr > F
133	1	286.2354264	286.2354264	10.06	0.0020
251	1	46.3540820	46.3540820	1.63	0.2050
37	1	25.5874098	25.5874098	0.90	0.3455
251*37	1	287.6302032	287.6302032	10.11	0.0020
219	1	66.4565051	66.4565051	2.34	0.1298
72	1	2.0298346	2.0298346	0.07	0.7900
219*72	1	348.9896261	348.9896261	12.26	0.0007
109	1	14.6215102	14.6215102	0.51	0.4753
165	1	53.3433569	53.3433569	1.87	0.1742
109*165	1	144.5430332	144.5430332	5.08	0.0265
309	1	0.2601904	0.2601904	0.01	0.9240
61	1	4.5273121	4.5273121	0.16	0.6909
309*61	1	243.7439569	243.7439569	8.56	0.0043
267	1	7.5845326	7.5845326	0.27	0.6069
278	1	0.0008712	0.0008712	0.00	0.9956
267*278	1	113.8508215	113.8508215	4.00	0.0484

Dependent Variable: PWE CSH00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	952.05640	476.02820	7.45	0.0008
Error	143	9139.74553	63.91430		
Corrected Total	145	10091.80193			

R-Square	Coeff Var	Root MSE	PWECH0 Mean
0.094340	15.06962	7.994642	53.05137

Source	DF	Type III SS	Mean Square	F Value	Pr > F
231	1	718.7415156	718.7415156	11.25	0.0010
223	1	489.5312322	489.5312322	7.66	0.0064

Dependent Variable: PWE TAH00

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	347.774446	347.774446	10.63	0.0014
Error	141	4613.948053	32.723036		
Corrected Total	142	4961.722499			

R-Square	Coeff Var	Root MSE	PWETH0 Mean
0.070091	20.06451	5.720405	28.51007

Source	DF	Type III SS	Mean Square	F Value	Pr > F
119	1	347.7744464	347.7744464	10.63	0.0014

Dependent Variable: PWE CSH01

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	246.297669	123.148834	7.83	0.0006
Error	142	2234.235672	15.734054		
Corrected Total	144	2480.533341			

R-Square	Coeff Var	Root MSE	PWECH1 Mean
0.099292	15.90262	3.966617	24.94317

Source	DF	Type III SS	Mean Square	F Value	Pr > F
271	1	99.8535044	99.8535044	6.35	0.0129
196	1	170.8423808	170.8423808	10.86	0.0012

Dependent Variable: PWE TCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	211.453430	105.726715	7.30	0.0010
Error	141	2041.342114	14.477604		
Corrected Total	143	2252.795544			

R-Square	Coeff Var	Root MSE	PWEHYB Mean
0.093863	10.72287	3.804945	35.48438

Source	DF	Type III SS	Mean Square	F Value	Pr > F
348	1	113.3741448	113.3741448	7.83	0.0059
165	1	89.8081258	89.8081258	6.20	0.0139

Dependent Variable: PWE TCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	659.098211	59.918019	5.22	<.0001
Error	118	1355.169577	11.484488		
Corrected Total	129	2014.267788			

R-Square	Coeff Var	Root MSE	PWEHYB Mean
0.327215	9.566156	3.388877	35.42569

Source	DF	Type III SS	Mean Square	F Value	Pr > F
348	1	69.9206526	69.9206526	6.09	0.0150
165	1	63.2243708	63.2243708	5.51	0.0206
235	1	14.9509942	14.9509942	1.30	0.2562
255	1	9.6480758	9.6480758	0.84	0.3612
235*255	1	92.3514332	92.3514332	8.04	0.0054
146	1	27.5269829	27.5269829	2.40	0.1243
2	1	17.4648074	17.4648074	1.52	0.2200
146*2	1	49.8760222	49.8760222	4.34	0.0393
311	1	21.2397096	21.2397096	1.85	0.1764
80	1	12.9482400	12.9482400	1.13	0.2905
311*80	1	135.8984742	135.8984742	11.83	0.0008

VITA

Jorge Luis Morán Maradiaga was born on August 5, 1973 in San Pedro Sula, Honduras. He graduated from the Escuela Agricola Panamericana, Zamorano, Honduras with a B.S. in agronomy in 1995. In August 2000, he received a M.S. in plant breeding from Texas A&M University. In August 2003, he received a Ph.D. in plant breeding from Texas A&M University.

Permanent mailing address:
Jorge Luis Morán
Colonia Loma Linda Sur # 3144
Tegucigalpa, Honduras

Telephone: (504)2397921